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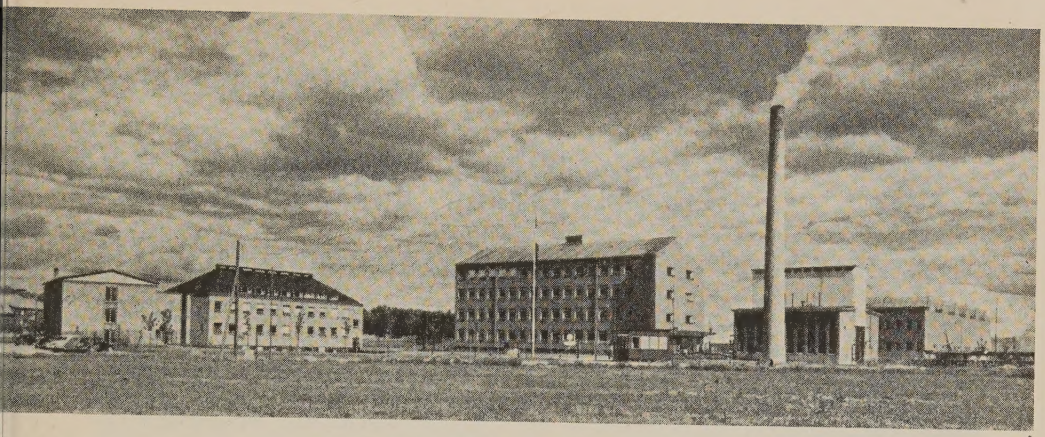

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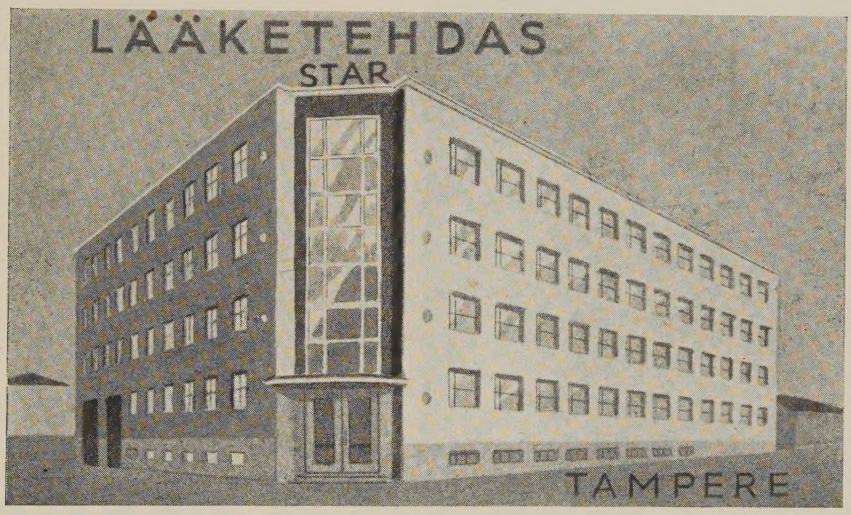

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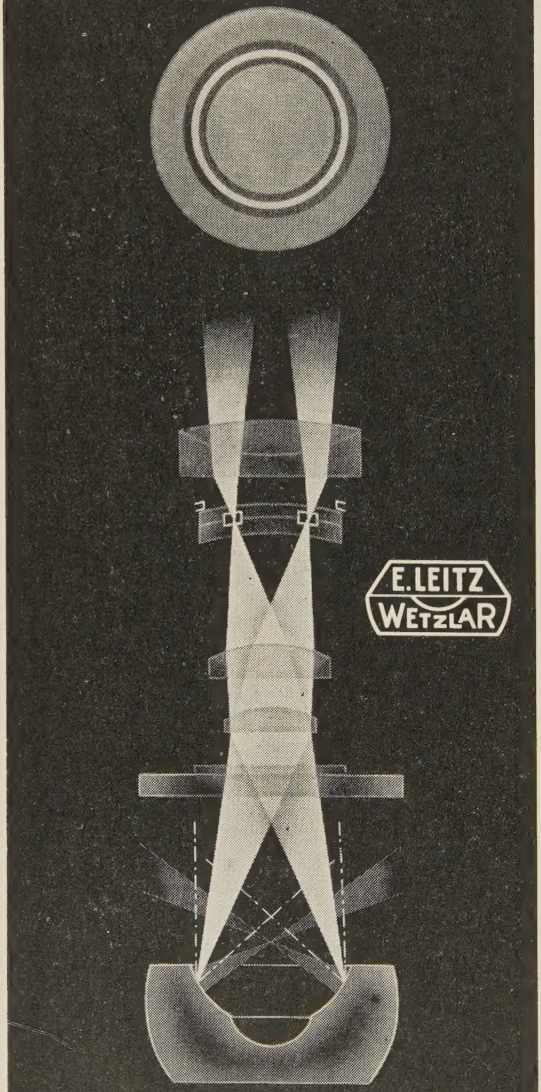

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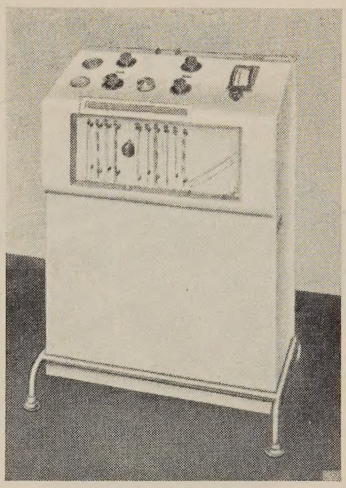

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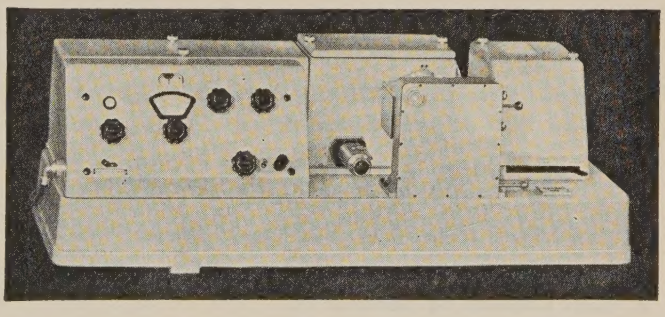

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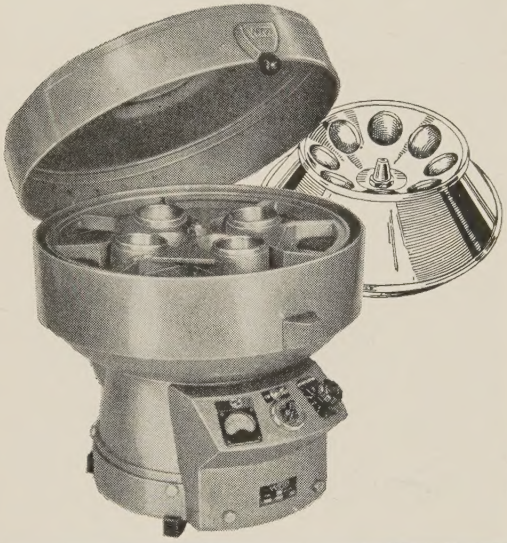

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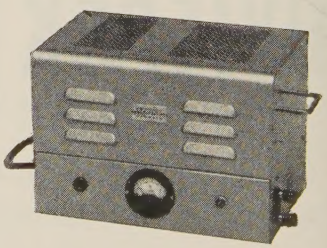

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THE EFFECT OF HEXAMETHONIUM BROMIDE ON THE PULMONARY CIRCULATION

By O. STORSTEIN AND H. TVETEN

From Nordland County Hospital, Stokmarknes, Norway

(Received for publication Febr. 18, 1954)

The ganglionic blocking agents hexamethonium bromide (HMB) and tetraethylammonium chloride (TEAC) are known to have the effect of reducing the blood-pressure, an effect which is utilized therapeutically in arterial hypertension (Smirk 1952, McMichael 1952 and Lyons & Love 1952). These agents also reduce the pulmonary artery pressure and the pulmonary capillary venous pressure (PCV). The reduction of the pressure in the pulmonary vessels is generally interpreted as an expression of a reduction in the vasomotor tone of the pulmonary vessels (Fowler, Westcott, Hauenstein, Scott & McGuire 1950, Greene & Bunnell 1950, Gilmore, Kopelman, McMichael & Milnes 1952, Halmágyi, Felki, Iványi, Zsótér, Tényi & Zsücs 1953[1] and Halmágyi, Iványi, Felkai, Zsótér, Tényi & Zsücs 1953[2]). Frisk, Hammarstrøm, Lagerström, Lagerlöf, Werkö, Björkenheim, Holmgren & Larson (1948) and Werkö, Frisk, Wade & Eliasch (1951), however, are of the opinion that the reduction of the pressure in the pulmonary vessels is due to a pooling of blood in the systemic blood vessels, with consequent reduction of the venous return.

The intention of this investigation is, if possible, to ascertain the site in the pulmonary circulation where the HMB effects the reduction in the pressures, and to find out whether the effect on the pulmonary blood vessels is a direct one or whether it is exerted through the systemic blood vessels, or in some other way.

MATERIAL AND METHOD

The material consisted of 14 subjects with various types of heart disease (Table I). Four subjects had normal pressures in the pulmonary artery, while the others showed elevated pressures, in some cases up to as much as 56 mm Hg mean pressure.

The day before the investigation the subjects were tested with HMB to ascertain what dose was necessary to bring about a moderate reduction of the blood pressure, the dose starting with 25 mg and being increased successively until a definite effect was noted. The next day the subjects were given a light breakfast. At 12.30 p. m. they

Table I. *Material for study of the effect of hexamethonium bromide on the pulmonary circulation.*

No.	Name	Sex	Age	Diagnosis	Height	Weight	Body surface area
1	L. I.	F	47	Mitral stenosis	1.57	62.5	1.62
2	B. A.	F	62	—»—	1.57	73	1.76
3	M. K.	M	58	Boeck's sarcoid	1.65	57	1.66
4	G. B.	F	61	Mitral stenosis	1.61	63.5	1.68
5	S. M.	F	45	—»—	1.49	56.2	1.49
6	A. H.	F	36	—»—	1.59	49.2	1.48
7	D. E.	F	49	—»—	1.64	55.2	1.60
8	E. T.	M	56	Hypertensive heart failure	1.70	67.1	1.78
9	J. H.	F	35	Mitral stenosis	1.65	64.7	1.72
10	H. H.	M	63	Hypertensive heart failure	1.67	67.6	1.76
11	J. O.	F	54	Mitral stenosis	1.61	66	1.70
12	H. K.	M	71	Arteriosclerotic heart dis.	1.75	72	1.87
13	P. E.	F	65	Thyreotoxic heart disease	1.59	61.5	1.63
14	J. S.	M	47	Hypertensive heart failure	1.82	88.9	2.12

came to the laboratory, where a cardiac catheter was introduced into the pulmonary artery under guidance of X-ray screening. Twenty minutes, on an average, following the introduction of the catheter, the pulmonary artery pressure, PCV and cardiac output were determined in the manner previously described (Storstein 1952). The mean pressures were determined with critical dampening (Tybjærg Hansen 1949). The pressure in the brachial artery was determined by the cuff method and the mean pressure calculated according to the formula: mean blood pressure = diastolic blood pressure + ⅓ pulse pressure. The previously determined dose of HMB was then injected subcutaneously. The determinations of the pressures were repeated at 5-minute intervals. After 20—30 minutes, the cardiac output was determined afresh and the catheterisation completed.

The statistical calculations have been carried out by Mr. Per Johannesen, M. A. In the tables

x denotes the observed value before treatment with HMB.

y denotes the observed value after treatment with HMB.

z denotes the difference between x and y ($z = x - y$).

$\bar{x}$, $\bar{y}$, $\bar{z}$ denote the arithmetic mean of x, y and z respectively.

s_x, s_y, s_z denote the sample standard deviation of x, y and z.

$s_{\bar{x}}$, $s_{\bar{y}}$, $s_{\bar{z}}$ denote the sample standard deviation of $\bar{x}$, $\bar{y}$ and $\bar{z}$ (standard deviation of mean).

The effect of treatment with HMB has been tested by means of "Student"s t- test (Cramér), where $t = \frac{\bar{z} \cdot \sqrt{n-1}}{s_z}$ follows the t distribution with $\vartheta = n-1$ degrees of freedom

RESULTS

The effect of HMB on the blood pressure, pulmonary artery pressure, PCV, heart rate, cardiac output, pulmonary arteriolar resistance (PAR), and total peripheral resistance (TPR) is shown in Tables II—VIII. The statistical calculations show a highly significant effect on the blood pressure, pulmonary artery pressure and cardiac output

Table II. *Effect of hexamethonium bromide on the blood pressure in 14 patients with heart failure.*

o.	Name	Sex	Mean BP before HMB	Dose	BP after HMB	Time
			Hg	mg	Hg	min.
1	L. I.	F	90 mm	25	90 mm	35
2	B. A.	F	113 »	25	90 »	30
3	M. K.	M	85 »	25	78 »	35
4	G. B.	F	100 »	25	90 »	35
5	S. M.	F	110 »	50	77 »	35
6	A. H.	F	90 »	25	68 »	35
7	D. E.	F	110 »	40	93 »	35
8	E. T.	M	143 »	50	127 »	35
9	J. H.	F	100 »	50	87 »	40
10	H. H.	M	120 »	25	87 »	35
11	J. O.	F	87 »	25	80 »	40
12	H. K.	M	93 »	50	70 »	35
13	P. E.	F	98 »	25	90 »	35
14	J. S.	M	147 »	100	80 »	35

$\bar{x} = 106.1$ $\bar{y} = 86.2$ $\bar{z} = 19.9$

$s_x = 18.8$ $s_y = 13.6$ $s_z = 16.6$

$s_{\bar{x}} = 5.0$ $s_{\bar{y}} = 3.6$ $s_{\bar{z}} = 4.3$

$t = 4.46$

$P < 0.001^{xxx}$

Table III. *Effect of hexamethonium bromide on pulmonary artery pressure in 14 patients with heart failure.*

o.	Name	Pulm. art. press. before HMB	Dose	P.A.P. after HMB	Time
		Hg	mg	Hg	min.
1	L. I.	53 mm	25	42 mm	35
2	B. A.	21 »	25	13 »	30
3	M. K.	22.5 »	25	20 »	35
4	G. B.	20 »	25	16.5 »	35
5	S. M.	33.5 »	50	27 »	35
6	A. H.	56 »	25	42 »	35
7	D. E.	23 »	40	20 »	35
8	E. T.	21 »	50	20 »	35
9	J. H.	34 »	50	23 »	40
10	H. H.	10 »	25	5 »	35
11	J. O.	36 »	25	32.5 »	40
12	H. K.	12 »	50	10 »	35
13	P. E.	10 »	25	7 »	35
14	J. S.	12 »	100	0.5 »	35

$\bar{x} = 26.0$ $\bar{y} = 19.9$ $\bar{z} = 6.1$

$s_x = 14.3$ $s_y = 12.3$ $s_z = 4.1$

$s_{\bar{x}} = 3.8$ $s_{\bar{y}} = 3.3$ $s_{\bar{z}} = 1.1$

$t = 5.37$

$P < 0.001^{xxx}$

Table IV. *Effect of hexamethonium bromide on PCV in 14 patients with heart failure.*

No.	Name	PCV before HMB	Dose	PCV after HMB	Time
		Hg	mg	Hg	min.
1	L. I.	43 mm	25	38 mm	25
2	B. A.	11.5 »	-	-	-
3	M. K.	3.5 »	-	-	-
4	G. B.	11.5 »	25	11 »	35
5	S. M.	15.5 »	50	8 »	30
6	A. H.	26 »	25	18 »	30
7	D. E.	18.5 »	40	8.5 »	32
8	E. T.	-	-	-	-
9	J. H.	27 »	50	22 »	40
10	H. H.	5.5 »	25	6 »	35
11	J. O.	30.5 »	25	19.5 »	35
12	H. K.	5 »	50	1.5 »	30
13	P. E.	6.5 »	25	5 »	30
14	J. S.	1 »	100	−2 »	32

$\bar{x} = 18.1$ $\bar{y} = 12.8$ $\bar{z} = 5.3$

$s_x = 14.3$ $s_y = 11.9$ $s_z = 3.8$

$s_{\bar{x}} = 4.3$ $s_{\bar{y}} = 3.6$ $s_{\bar{z}} = 1.1$

$t = 4.41$

$0.01 > P > 0.001^{xx}$

Table V. *Effect of hexamethonium bromide on heart rate in 14 patients with heart failure.*

No.	Name	Heart rate before HMB	Dose	Heart rate after HMB	Time
			mg		min.
1	L. I.	70	25	90	30
2	B. A.	-	-	-	-
3	M. K.	80	25	80	35
4	G. B.	80	25	80	35
5	S. M.	-	-	-	-
6	A. H.	92	25	80	35
7	D. E.	-	-	-	-
8	E. T.	64	50	64	35
9	J. H.	92	50	92	35
10	H. H.	66	25	80	35
11	J. O.	80	25	80	40
12	H. K.	54	50	43	35
13	P. E.	66	25	58	35
14	J. S.	105	100	92	35

$\bar{x} = 77.2$ $\bar{y} = 76.3$ $\bar{z} = 0.9$

$s_x = 14.3$ $s_y = 14.6$ $s_z = 9.9$

$s_{\bar{x}} = 4.3$ $s_{\bar{y}} = 4.4$ $s_{\bar{z}} = 3.0$

$t = 0.29$

$0.80 > P > 0.70$

Table VI. *Effect of hexamethonium bromide on cardiac index in 14 patients with heart failure.*

No.	Name	C.I. before HMB	Dose	C.I. after HMB	Time
		lit/min/m²	mg	lit/min/m²	min.
1	L. I	3.0	25	2.2	25
2	B. A.	1.9	25	1.7	20
3	M. K.	3.0	25	2.8	27
4	G. B.	2 5	25	1.7	27
5	S. M.	2.1	50	2.1	26
6	A. H.	3.4	25	2.8	25
7	D. E.	3.8	40	2.9	27
8	E. T.	3.0	50	2.9	30
9	J. H.	3.2	50	2.6	31
10	H. H.	3.0	25	1.9	30
11	J. O.	2.9	25	2.3	33
12	H. K.	2.2	50	1.8	27
13	P. E.	2.8	25	2.2	20
14	J. S.	2.8	100	1.8	25

$\bar{x} = 2.83$ $\bar{y} = 2\ 27$ $\bar{z} = 0.56$

$s_x = 0.49$ $s_y = 0.44$ $s_z = 0.33$

$s_{\bar{x}} = 0.13$ $s_{\bar{y}} = 0.12$ $s_{\bar{z}} = 0.09$

$t = 6.13$

$P < 0.001^{xxx}$

Table VII. *Effect of hexamethonium bromide on pulmonar arteriolar resistanc in 14 patients with heart failure.*

No.	Name	P.A.R. before HMB	Dose	P.A.R. after HMB	Time
		dynes sec.cm^{-5}	mg	dynes sec.cm^{-5}	min.
1	L. I.	162	25	89	25
2	B. A.	230	-	-	-
3	M. K.	316	-	-	-
4	G. B.	167	25	153	35
5	S. M.	461	50	487	30
6	A. H.	484	25	470	30
7	D. E.	59	40	198	32
8	E. T.	-	-	-	-
9	J. H.	101	50	18	40
10	H. H.	72	25	24	35
11	J. O.	249	25	266	35
12	H. K.	137	50	200	31
13	P. E.	26	25	44	30
14	J. S.	150	100	147	32

$\bar{x} = 174.3$ $\bar{y} = 184.2$ $\bar{z} = 9.7$

$s_x = 154.3$ $s_y = 160.2$ $s_z = 54.9$

$s_{\bar{x}} = 46.5$ $s_{\bar{y}} = 48.3$ $s_{\bar{z}} = 16.5$

$t = 0.56$

$0.60 > P > 0.50$

calculated as cardiac index (C. I.). There is also a significant effect on the PCV-pressure, whilst the effect on the heart rate, PAR and TPR is not significant.

DISCUSSION

HMB causes a definite reduction in the blood pressure. There is at the same time a fall in the cardiac output. This reduction of the cardiac output may be caused either by dilatation of the peripheral blood vessels, with pooling of blood in the systemic veins and capillaries and subsequent reduction of the venous return (Frisk *et al.* 1948, Werkö *et al.* 1951), or it may be due to a reduced sympathetic tone of the heart muscle, with decreased contractility (Green 1953). In either case the reduction of the cardiac output will be more distinct the greater the gang lionic blocking effect which follows the in jected dose of HMB (Werkö *et al.* 1951 Grob, Scarborough, Kattus & Langfor 1953.

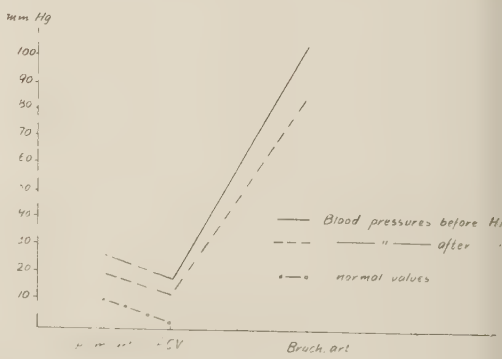

Fig. 1. Mean pressure in pulmonary artery, PCV and brachial artery before and after HMB.

able VIII. *Effect of hexamethonium* omide *on total peripheral resistance in 14 patients with heart failure.*

	Name	T.P.R. before HMB	Dose	T.P.R. after HMB	Time
		dynes sec.$^{-}$cm^{5}	mg	dynes sec.cm^{-5}	min.
	L. I.	1462	25	1996	25
	B. A.	2737	25	2497	25
	M. K.	1415	25	1332	35
	G. B.	1960	25	2497	35
	S. M.	2818	50	2126	30
	A. H.	1453	25	1175	30
	D. E.	1436	40	1609	35
	E. T.	2116	50	1944	35
	J. H.	1448	50	1335	40
	H. H.	1926	25	1865	35
	J. O.	1350	25	1639	35
	H. K.	1822	50	1636	30
	P. E.	1700	25	1843	30
	J. S.	2000	100	1720	35

$\bar{x}$ = 1831.0 $\bar{y}$ = 1801.0 $\bar{z}$ = 30.6

s_x = 457 s_y = 384 s_z = 324

$s_{\bar{x}}$ = 122.2 $s_{\bar{y}}$ = 102.7 $s_{\bar{z}}$ = 86.7

t = 0.34

0.80 > P > 0.70

Fig. 1 shows the effect of HMB on the monary artery pressure, PCV and brachial ery pressure. It will be seen that the fall the pressure following HMB is somewhat eater in the systematic arteries than in pulmonary blood vessels. The fall in essure in the pulmonary artery and PCV the same.

Simultaneous measurement of the PCV-essure and the pressure in the left atrium, ether *via* the left main bronchus (Epps & ller 1953), by direct puncture or through atrial septal defect (Werkö, Varnauskas, iasch, Lagerlöf, Senning & Thomasson 53), indicates that the PCV-pressure is ntical with the left artial pressure.

If HMB exerts its effect on the tone of pulmonary arterioles we would expect a greater fall in the pulmonary artery pressure than in the PCV-pressure. Since the fall of the pressure is the same we may conclude that the effect of HMB, which we have observed on the pulmonary artery pressure, is *not* due to any active dilatation of these vessels, but that it is an expression of a reduction of the pressure in the left atrium and left ventricle.

The fall of the pressure in the pulmonary circulation following HMB may also be connected with the reduced cardiac output, since there is a distinct relation between blood flow and pulmonary artery pressure both in normal individuals and in subjects with lung dis eases: the pressure increases with the increase in blood flow (Storstein 1952).

The effect observed on the pulmonary circulation of various agents acting on the autonomic nervous system may be explained by three different hypotheses:

1) Active effect on the pulmonary blood vessels. Fowler *et al.* (1950) are of the opinion that the fall in the pulmonary artery pressure, which they have observed following TEAC in normal individuals and in subjects with arterial hypertension and lung diseases, is due, at any rate in part, to a direct effect on the pulmonary blood vessels. Greene & Bunnell (1950) have made similar observation with TEAC and are of the opinion that the fall in the pressure must be explained by a reduction of the vasomotor tone in the pulmonary artery, as the effect on the cardiac output was insufficient to explain the reduction of the pressure. Halmágyi *et al.* (1953 I & II) have investigated the effect on the pulmonary artery pressure of various agents: Dihydroergotamine gave a slight increase in the pulmonary artery pressure

and systemic blood-pressure, whilst sodium nitrite, TEAC, dibenamine and sleep caused a fall in the pressure, both in the systemic and pulmonary circulation. The effect on the heart rate and the cardiac output was slight. They also assumed a direct effect on the pulmonary artery pressure, while the change in the pressure was not always parallel in the systemic and pulmonary circulations. In objection to their methods one might point out that they use the systolic pressure in calculating the resistance both in the systemic and in the pulmonary circulation. Gilmore *et al.* (1952) also ascribe the effect they observed on the pulmonary artery pressure following HMB to a liberation of vasoconstricting impulses. This liberation became still more apparent following a 30—45 degree raising of the head, when the pressures fell most and there was an increase in the "cardio-pulmonary blood volume". Thaddeus, May, Bennett, Kobayashi & Gregory (1953), in experimental arterial hypertension in dogs, have observed an increase in the pulmonary artery pressure following angiotonine, adrenaline and noradrenaline and a fall in the pressure following TEAC and theophylline. They also ascribe this change in the pressure to a direct constricting or dilating effect on the pulmonary vessels.

2) Passive effect on the pulmonary vessels of pressure changes in the systemic circulation. The proponents of this hypothesis emphasize the paucity of autonomic innervation of the pulmonary blood vessels (Brenner 1935), and the improbability of any pronounced effect on the pulmonary vessels of agents acting on the autonomic nervous system. It is, moreover, important to bea in mind that the pulmonary circulation i a low pressure system, the pressures bein from ⅙ to ⅛ of the pressures in the systemi circulation. The upper normal limit for th pulmonary artery pressure with the angl of Louis as a reference point is 16/6—10 mm Hg (Storstein 1952). A change of pressur in the systemic circulation will accordingl influence the height of pressure in the pul monary circulation. The chief proponent o this view-point is Hamilton (1951). H ascribes the effect on the pulmonary arter pressure, which he has observed in dog after amylnitrite, mecholyl and adrenaline to this "back-pressure" effect. Fowler, West cott, Scott & McGuire 1951) also ascrib the increase in the pressure, which they hav observed in the pulmonary artery and PCV after nor-adrenaline, to a "back-pressure effect from the left atrium. Rakita & San cetta (1953) in 10 normal individuals ob served a fall in brachial artery and pul monary artery pressures, with no change i cardiac output following 15 to 120 mg HMI i.v. They ascribe the fall in the pressures t a reducing effect of HMB on the total peri pheral resistance.

3) Bronchomotor tone. Rodbard (1953 puts forward the view that the bronch react to a change in the tone of the auto nomic nervous system. Increase of the vago tonus promotes contraction and increase o sympathetic tone dilatation of the bronch Through contraction the calibre of th bronchi will decrease, and the alveolo capillary surface will also decrease, with sub sequent increased resistance to the bloo flow. On the other hand dilatation of th

nchi will lead to an increased alveolo-illary surface, with lesser resistance to the od flow. This viewpoint may explain the ssure increase in pulmonary emphysema l bronchial asthma. In asthma there is an r trapping" in the bronchi and alveoles, ich is easily demonstrated by spirography eslie 1952). According to this hypothesis nts acting on the autonomic nervous tem will exert their effect on the pul-nary artery pressure through a con-cting or dilating effect on the bronchi. is may be the explanation of the pheno-non that certain pharmacological agents y have an opposite effect on the blood ssure in the systemic circulation from that the pressure in the pulmonary circulation. dbard (1953) in experiments on dogs owing acethylcholine, has observed an rease of the diastolic pressure in the pul-nary artery simultaneously with a fall in ssure in the systemic circulation. On the er hand, in the case of chickens following enaline, he has observed a fall of the stolic pressure in the pulmonary artery ultaneously with an increase of the pres-e in the systemic arteries. This opposite ct on the pressures in the systemic and pulmonary circulation is not explainable the passive hypothesis, but is easily lained by the effect on the bronchi. As ule, however, adrenaline does result in an rease in the pulmonary artery pressure, observed in experiments with dogs amilton 1951, Thaddeus *et al.* 1953).

While change in bronchomotor tone may lain changes of pressure in the pulmonary culation in bronchial asthma, it will not lain the change of pressure in the pul-monary circulation which we have observed following the use of HMB. This change of pressure is best explained by the passive hypothesis. Admittedly, we have not observed the "breathing pattern" in our subjects, but we did not observed any change in the rate or depth of respiration. Nor was there any change in the ventilation following HMB.

The only stimulus which is definitely known to contract the pulmonary blood vessels is anoxia (v. Euler & Liljestrand 1946, Motley, Cournand, Werkö, Himmelstein & Dresdale 1947, Nisell 1950, Westcott, Fowler, Scott, Hauenstein & McGuire 1951, Storstein 1952). This contracting effect is unaffected by agents which act on the autonomic nervous system (Logaras 1947, Nisell 1950), nor is it to be explained by its effect on the bronchi, since anoxia dilates the the bronchi (Nisell 1950).

SUMMARY

In 14 subjects with various type of heart disease hypotensive doses of hexamethonium bromide produced a significant fall of the blood pressure in the systemic arteries, pulmonary artery and pulmonary capillary, as well as a significant fall in the cardiac output. There was no effect on the heart rate or on the resistance in the systemic or pulmonary circulation.

The fall in the cardiac output can be explained by the dilatation in the systemic blood vessels, with pooling of blood in these vessels, or by a reduction of the sympathetic tone of the heart muscle. As the fall of pressure is identical in the pulmonary artery and pulmonary capillary, it is concluded that

hexamethonium bromide has no selective dilating effect on the pulmonary artery. The fall in pressure must be explained by the fall in pressure in that part of the circulation which lies before the pulmonary circulation, *i. e.* the left ventricle and the left atrium.

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ISOTOPIC STUDIES ON THE MECHANISM OF NEGATIVE NITROGEN BALANCE PRODUCED BY IMMOBILIZATION

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It is a well-known fact that bed-patients often show negative N-balance (Pollack & Halpern 1951). Deitrick, Whedon & Shorr (1948) have observed the same phenomenon in healthy, immobilized subjects. The exact mechanism that causes this state of nitrogen disequilibrium has not been ascertained. It is not known whether the negative N-balance is due to stoppage of the tissue repair while tissue breakdown continues at an ordinary rate, or whether the rate of tissue breakdown is increased and the rate of protein synthesis is normal. In the present paper an attempt has been made to elucidate this problem by means of excretion data for N^{15} from subjects fed N^{15}-labelled glycine.

Olesen, Heilskov & Schønheyder (1954) have recently published an investigation on the nitrogen interaction in normal humans in nitrogen balance. They measured the N^{15}-excretion in urine for 360 hours after the administration of N^{15}-glycine and found that the N^{15}-abundance in excess in urine, a, followed the equation:

$$a = a_1 e^{-B_1 t} + a_2 e^{-B_2 t} + a_3 e^{-B_3 t} \quad (1)$$

where t = time in hours and a_i and B_i are empirical constants.

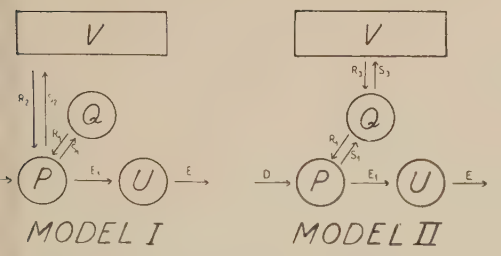
They found that the cumulative excretion of N^{15} in urine within the time period mentioned could be rendered by the integrate expression of equation (1):

$$\frac{\lambda_E}{\lambda_o} = A_1 (1 - e^{-B_1 t}) + A_2 (1 - e^{-B_2 t}) + A_3 (1 - e^{-B_3 t}) \quad (2$$

where λ_E = the amount of N^{15} in exces excreted in urine up to time t, λ_o = th amount of N^{15} in excess fed at zero tim (minus the amount of N^{15} in excess excrete in feces). A_i and B_i are constants.

In the work mentioned above the differer possibilities of nitrogen interaction in th organism were discussed, certain assump tions being made for the models given i Fig. 1. If the amount of N^{15} (λ_o) is adde to the flow D, and the amount of N^{15} (λ leaves the system by the flow E, it wa found by mathematical analysis of the kine tics of distribution of N^{15} in such system that the expressions for N^{15}-abundance (a and (λ_E/λ_o) in the flow E were of a shap analogous to equations (1) and (2).

The suppositions for the mathematic treatment of the model systems are as fo lows. P, Q and U are homogeneous nitroge pools and V is a blind nitrogen pool, *i.* the amount of nitrogen which is exchange in it during the experiment is negligible proportion to its total nitrogen conten



. 1. Models for the nitrogen interaction in the anism. P, Q, U and V are nitrogen pools. , E1, E, S1, R1, S2, R2, S3 and R3 are flows of nitrogen.

Table I. *k-values expressed in terms of A_i and B_i.*

$$k_1 = B_2 + B_3 - k_3$$

$$k_3 = B_1B_2\frac{A_1(B_3 - B_1) + A_2(B_3 - B_2)}{A_1B_1(B_3 - B_1) + A_2B_2(B_3 - B_2)}$$

$$k_2k_4 = k_1k_3 - B_2B_3$$

$$k_5 = B_1$$

$$k_6 = \frac{1}{B_1}\left[A_1B_1(B_3 - B_1) + A_2B_2(B_3 - B_2)\right]$$

The k-values denote the coefficients in the differential equations set up for the models. A_i and B_i are constants which fit the empirical equations.

rthermore, there is proportionality be- en the amounts of nitrogen in the pools the flows leaving these pools. The hors outlined a method by which it was sible, on the basis of the empirical con- nts, A_i and B_i, obtained from experi- nts on subjects in nitrogen balance, to pute the magnitude of the pools P, Q U, and rates of the N-flows S_1, R_1, S_2, S_3 and R_3. The principle followed in calculation of these parameters is first solve the differential equations arrived at the changes in the amount of N^{15} per time in each of the pools P, Q and U, as to obtain expressions for the coeffi- nts of the differential equations in terms he experimental constants A_i and B_i, Table I. Then the parameters are cal- ted from the coefficients, assuming that P, Q and U-pools, and the organism as hole, are in a quasi-stationary state, *i. e.* $= \overline{S}_1$; $\overline{R}_2 = \overline{S}_2$; $\overline{R}_3 = \overline{S}_3$ and $\overline{E} = \overline{D}$. The meters mentioned here indicate average es for 24 hours. The authors believe P-pool to include the mixture of amino s in the organism which are renewed from the diet and from the breakdown of nitrogenous compounds in the V- and the Q-pools. From the P-pool the U-pool, which contains the nitrogenous compounds in the organism which are excreted in the urine, is renewed. The Q-pool comprises the metabolically active nitrogenous constituents in the organism which have a short half-life time (*e. g.* serum proteins and proteins in internal organs), while the V-pool contains metabolically inert nitrogenous constituents in the organism (chiefly muscle and connective tissue proteins).

First approximations estimates of A_i and B_i are calculated on the basis of the logarithmic graph of the experimental results. The final choice of constants was not considered satisfactory until the A_i- and B_i -values chosen could render the experimental results both in the logarithmic and in the cumulative plottings.

Sprinson & Rittenberg (1949 b), Hoberman (1950) and San Pietro & Rittenberg (1953) have investigated problems of similar type, but the assumptions made by these authors, the mathematical treatment of their systems and the experimental procedures deviate from ours.

In two of the cases previously examined where the N^{15}-excretion after administration of N^{15}-labelled glycine was checked for 360 hours during nitrogen balance, similar experiments were performed in which the subjects were immobilized in bed for a considerable time and showed a negative nitrogen balance.

In the case of the immobilized persons we also find that equations (1) and (2) are able to represent the N^{15}-excretion in a satisfactory way. By employing the empirical constants obtained in the experiments last mentioned it is possible to elucidate more closely the mechanism of the negative nitrogen balance during immobilizing confinement to bed.

EXPERIMENTAL

The investigation was carried out on 3 normal subjects *HU, RB* and *ES*. During the experiments they were given a well-defined diet which each day contained the same amounts of protein, fat, carbohydrate and salts. Furthermore, all breakfasts (at 8 a.m.) were exactly the same as were also dinner (at noon) and supper (at 6 p.m.).

The amount of nitrogen in the diet was determined daily by Kjeldahl-analysis and the same was done with all urine portions. Furthermore, N-determinations were performed in pooled specimens of feces from 4—7 days. Between 1.00 and 1.05 g of nitrogen were found per day. The N-balances of the subjects under normal and under immobilized conditions are given in Table II. As the N-balance during immobilization varies somewhat from one day to another the daily N-balances for these periods are indicated in Fig. 2.

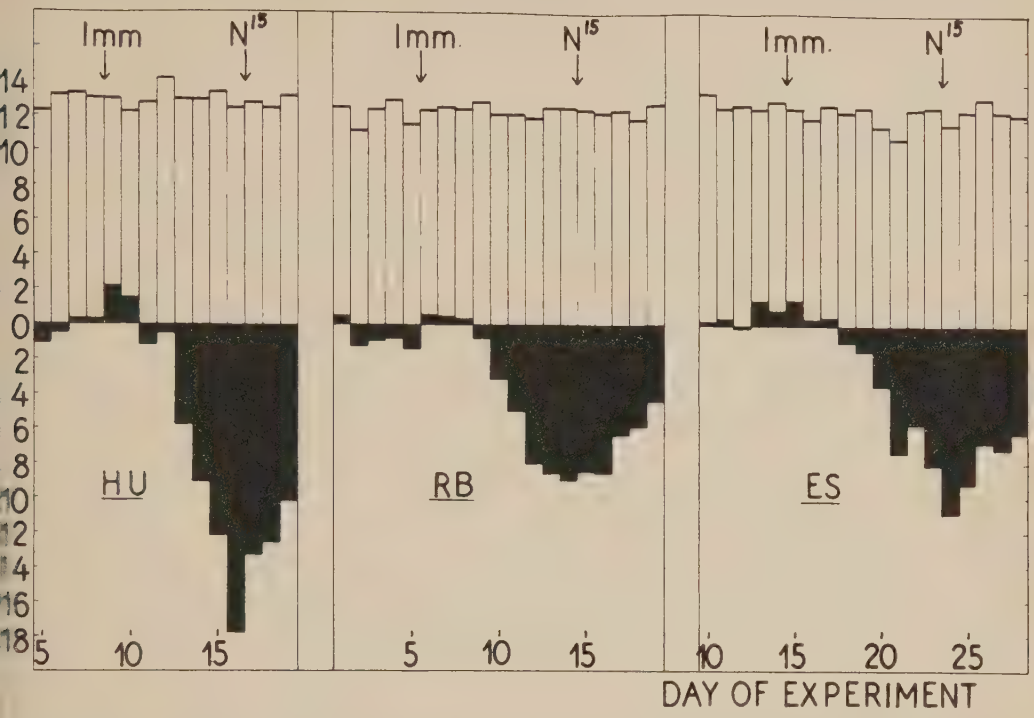
Table II. *Nitrogen balance during th experimental periods.*

		$\bar{D}$ Dietary N g per day	$\bar{E}$ Urinary + fecal N g per day	$\bar{E}-\bar{I}$ g pe day
HU	normal (9)....	12.76±0.42	12.65±1.26	
	imm. (3)	12.88±0.35	24.04±1.32	11.2
RB	*RB-I*-nor. (16) .	12.17±0.43	12.13±0.68	
	RB-II-nor. (10).	12.17±0.38	11.65±1.05	
	RB-imm. (5) ..	12.57±0.30	19.21±1.81	6.6
ES	normal (20) ...	12.46±0.51	12.80±1.04	
	imm. (5)	12.45±0.54	20.45±1.55	8.0

The figures represent mean values over period of the number of days indicated in the parenthese

The glycine preparations used were pre pared according to the method of Schoen heimer & Ratner (1939). The abundanc of N^{15} in excess for the glycine which wa used in Exps. *HU* and *RB* was 34.13 ato per cent, and in Exps. *ES* 35.93 atom pe cent. About 800 mg of the N^{15}-glycine, care fully weighed, was given orally at 8 p.m It can be assumed that the amount of N in excess which can be determined in fece after the administration of N^{15}-glycine (1.1–2.8 per cent of the amount given per os arises from non-resorbed N^{15}. Consequentl the amount of N^{15} in excess, λ_o, which wa introduced into the experimental subjec was computed by substracting the amoun found in feces during the first 8 days aft the administration from the excess-N^{15} a ministered per os. After this time the N excretion in feces was negligible.

After the administration of glycine th urine was collected first at short, later on longer intervals. On each urine samp nitrogen determinations and mass spectr metric analyses of N^{15}-abundance in th urinary nitrogen were performed.



g. 2. Parts of the study on daily nitrogen balance in the three experimental subjects. Nitrogen intake charted upwards from the zero line and nitrogen output (urine + feces) is charted downwards from e intake line. Positive and negative balance is indicated in black over and under the zero line. *Imm.* immobilizing bed rest was begun. At N^{15} glycine labelled with N^{15} was administered and the excretion experiment was started.

he preparation of gas samples for mass ctrometric analysis was carried out in the nner described by Sprinson & Rittenberg 49 a). N^{15}-abundances were determined means of a Metropolitan Vickers Mass ctrometer, MS-3, using the double collec- method. Usually a quadruple determina- was performed on each sample. The dard deviation on the average of druple determinations was about 0.1 per (Heilskov, Olesen, Schønheyder & rner, 1953). For each sample of urine feces the N^{15}-abundance in excess was ulated above the abundance found in the urine and feces from the same subjects before the experiment. In some of the cases N^{15}-glycine had been administered in preceding experiments, and in these a small slightly decreasing N^{15}-abundance in excess could still be measured in the urine before the new administration of N^{15}-glycine. This has been considered in calculating the N^{15}-abundances in excess.

During the experiments which show the N^{15}-excretion in nitrogen equilibrium the subjects attended their daily work. Regarding the experiments the following details should moreover be given.

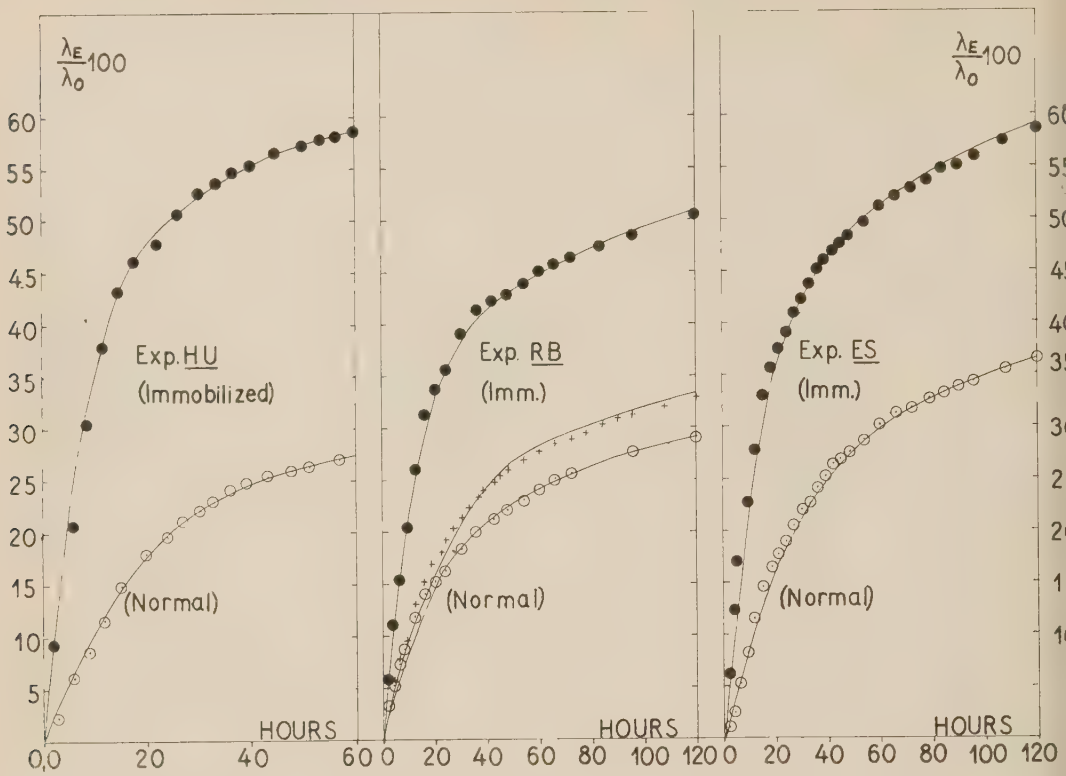


Fig. 3. Effect of immobilization on the cumulative urinary N^{15}-excretion in excess. *RB—I:* + —— +. *RB—II:* o —— o.

Exps. HU. Aged 21 years. After *HU* had been given the diet for 6 days the *Exp. HU-normal* was carried out. On the 9th day of the experiment he was put to bed and placed in a bivalved plaster of Paris bandage extending from the umbilicus to the toes. After 5 days of immobilization a negative N-balance was observed, which increased and attained a maximum on the 8th day. On the 9th day the second isotope experiment was started. Weight before immobilization 59.0 kg, after immobilization 58.0 kg.

Exps. RB. Aged 20 years. Here the immobilization experiment was performed first. *RB* was given the diet for 5 days and then put to bed with a plaster of Pari bandage immobilizing both lower extremities 5 days later a negative N-balance began t appear. Maximum was reached in 9 days On the 10th day of immobilization *Exp. RB imm.* was started. Weight before immobiliza tion 68.4 kg, after immobilization 66.5 kg *Exps. RB-II* and *RB-I* (both in nitroge equilibrium) were carried out 74 and 11 days, respectively, after the time when *Exp RB-imm.* was started.

Exps. ES. Aged 23 years. *Exp. ES normal* was carried out after *ES* had bee on the diet for 5 days. On the 15th day o the experiment he was put to bed with

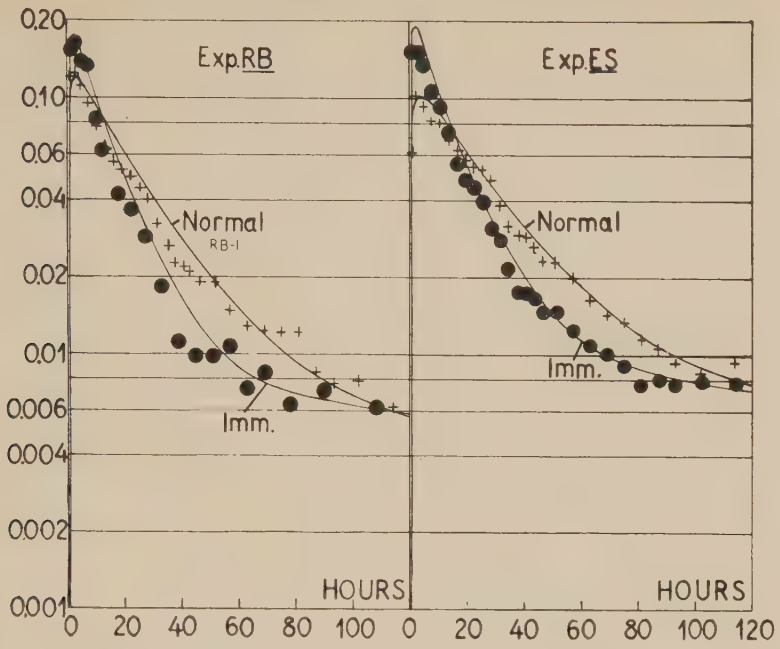


Fig. 4. Semilogarithmic plots of urinary N^{15}-abundance in excess in relation to time.

ster of Paris bandage which included h lower extremities. 6 days later a nega- e N-balance occurred which reached its ximum on the 10th day of immobilization. the same day *Exp. ES-imm.* was started. ight before immobilization 63.9 kg, after mobilization 63.1 kg.

RESULTS AND DISCUSSION

n the 3 experimental subjects a marked ative N-balance appeared on the diet ch did maintain N-equilibrium when the jects were not immobilized and went to k. The negative N-balance occurred after g of about 5 days and reached a maxi- n about the 10th day of immobilization. en there was an unmistakable decline in negative N-balance. We have not, in the ature on the subject, come upon any explanation of this peculiar sequence which appears very clearly from Fig. 2. Deitrick, Whedon & Shorr (1948) also observed a certain lag between the beginning of immobilization and the entrance of negative N-balance and found the maximum nitrogen loss to occur during the second week of immobilization.

In the preliminary experiment on *HU* an obvious difference, cf. Fig. 3, was found between the cumulative N^{15}-excretion during the two different experimental conditions, the N^{15}-excretion being much larger during immobilization than under normal conditions. As the excretion was examined for a shorter period than in the later experiments we refrained from applying to these results the same mathematical treatment as to the subsequent ones.

Table III. λ_0, *E and empirical constants in experiments on normal and immobilized subjects.*

	ES		*RB*		
	Normal	Immobilized	RB-I Normal	RB-II Normal	Immobilized
$\lambda_0 < \frac{\text{m.eqv.}}{\text{g}}$ ..	3.64	3.64	3.55	3.50	3.58
..	0.0548	0.0548	0.0532	0.0525	0.0537
E g N/hr	0.489*	0.809**	0.448*	0.447*	0.759**
A_1	0.288	0.462	0.283	0.214	0.414
A_2	0.347	0.548	0.253	0.291	0.402
A_3	−0.0122	−0.0340	−0.0162	−0.0131	−0.0317
B_1/hr.....	0.0399	0.0750	0.0400	0.0550	0.0805
B_2/hr.....	0.0025	0.0030	0.0025	0.0032	0.0032
B_3/hr.....	1.01	1.06	1.02	0.97	1.09
a_1	0.1200	0.2200	0.1560	0.1290	0.2200
a_2	0.0090	0.0104	0.0071	0.0102	0.0085
a_3	−0.1290	−0.2304	−0.1631	−0.1392	−0.2285

* mean values for 360 hr. period.
** mean values for 120 hr. period.

The examinations on *RB* and *ES* also showed a markedly greater cumulative excretion of N^{15} during immobilization than under normal conditions (Fig. 3). In Fig. 4 are plotted the abundance values of urinary N^{15} in excess found in the experiments. For the sake of clarity only one of the experiments on *RB* under normal conditions is given here. It can be seen that during immobilization both *RB* and *ES* reach a higher maximum and a steeper fall of N^{15}-abundance in urine than under normal conditions. It should be mentioned that the same phenomenon was found for *HU*.

The experimental results tend to show that during immobilization, where the N-balance is negative, the organism treats the dietary nitrogen in a different way than under normal conditions.

The theoretical curves which are drawn in Figs. 3 and 4 have been constructed by means of equations (2) and (1), employing the empirical constants a_i, A_i and B_i whic are given in Table III. It appears that, bot under normal conditions and with immo bilized subjects, there is a satisfactory agree ment between the constructed curves an the points plotted from the experiments i each of the two graphs. The curves for th experimental subjects, when they are nor mal, have previously (Olesen, Heilskov & Schønheyder, 1954) been published in the entirety (360 hours). In this paper only th data for the first 120 hours have been in cluded because in the immobilization exper ments only this period was dealt with.

In experiments on subjects in nitroge equilibrium the empirical constants wer used for calculating the k-values accordin to Table I, as mentioned in the introductio From these k-values the pool sizes and rat of flows were then calculated on the bas of the relationships in Table IV.

Table IV. *Parameter determinations in terms of k-values.*

	Model I	Model II
$\bar{P}$	$\frac{\bar{E}}{k_6}$	$\frac{\bar{E}}{k_6}$
$\bar{Q}$	$\frac{k_2 k_4}{k_3^2 k_6}\bar{E}$	$\frac{k_6}{k_2 k_4}\left(\frac{k_1}{k_6}-1\right)^2\bar{E}$
$\bar{U}$	$\frac{\bar{E}}{k_5}$	$\frac{\bar{E}}{k_5}$
$\bar{S}_1$	$\frac{k_2 k_4}{k_3 k_6}\bar{E}$	$\left(\frac{k_1}{k_6}-1\right)\bar{E}$
$\bar{R}_1$	$\bar{S}_1$	$\bar{S}_1+\bar{E}-\bar{D}$
$\bar{S}_2$	$\left(\frac{k_1}{k_6}-1-\frac{k_2 k_4}{k_3 k_6}\right)\bar{E}$	
$\bar{R}_2$	$\bar{S}_2+\bar{E}-\bar{D}$	
$\bar{S}_3$		$\left[\frac{k_3 k_6}{k_2 k_4}\left(\frac{k_1}{k_6}-1\right)-1\right]\left(\frac{k_1}{k_6}-1\right)\bar{E}$
$\bar{R}_3$		$\bar{S}_3+\bar{E}-\bar{D}$

For organism in nitrogen equilibrium, *i. e.* $\bar{E}=\bar{D}$, is $\bar{R}_2=\bar{S}_2$ (in Model I), $\bar{R}_1=\bar{S}_1$ and $\bar{R}_3=\bar{S}_3$ (in Model II).

If one wants to use the empirical constants m experiments on subjects in negative N-ance in similar way as in the experi-nts on normal persons it is necessary to stulate that the assumptions which form basis of the calculations under normal nditions are also valid for persons who are in nitrogen balance. Assuming that during nobilization there is also proportionality ween the sizes of each of the homogenous ols, P, Q and U, and the flow from it, same relationships are obtained between coefficients in the differential equations l the empirical constants as those shown Table I. In the derivation of the parameter pressions for the organisms in nitrogen ilibrium we assumed a quasi-stationary te in all the pools. When deriving the analogous expressions for organisms in nitrogen disequilibrium we assume that there is only a quasi-stationary state in the pools P, Q and U, but not in the V-pool. This means that the negative nitrogen balance (E—D) appears at the expense of the V-pool. The expressions which are used in the calculations of the parameters in immobilization experiments will, with the following exceptions for rates of breakdown, be the same as those employed in nitrogen balance experiments.

Model I. Here we have: $D + R_2 + R_1 = S_1 + S_2 + E_1$, as an expression of the quasi-stationary state in the P-pool. As $R_1 = S_1$, and $E_1 = E$, expressing a quasi-stationary state in the Q- and U-pools, we get $R_2 = S_2 + E - D$.

Table V. *Mean values (over 24 hours) of pool sizes and rates of flows in the experimental subjects under normal and immobilized conditions, computed according to model I and model II by help of E and the empirical constants in Table III and the formulae in Table IV.*

		Model I			Model II		
		Normal			Normal		
		RB-I	RB-II	RB-Immob.	RB-I	RB-II	RB-Immob.
$\bar{P}$	g N	1.6	2.1	1.7	1.6	2.1	1.7
$\bar{Q}$	»	156	157	148	385	311	222
$\bar{U}$	»	9.7	8.1	9.4	9.7	8.1	9.4
$\bar{S}_1$	g N/24 hr	17.2	27.1	22.2	27.0	38.2	27.2
$\bar{R}_1$	»	17.2	27.1	22.2	27.0	38.2	32.8
$\bar{S}_2$	»	9.8	11.1	5.0	-	-	-
$\bar{R}_2$	»	9.8	11.1	10.6	-	-	-
$\bar{S}_3$	»	-	-	-	15.4	15.6	6.2
$\bar{R}_3$	»	-	-	-	15.4	15.6	11.8

		ES-Normal	ES-Immob.	ES-Normal	ES-Immob.
$\bar{P}$	g N	1.6	1.7	1.6	1.7
$\bar{Q}$	»	162	148	295	154
$\bar{U}$	»	12.3	10.7	12.3	10.7
$\bar{S}_1$	g N/24 hr	20.2	23.6	27.3	23.5
$\bar{R}_1$	»	20.2	23.6	27.3	30.4
$\bar{S}_2$	»	7.1	0.5	-	-
$\bar{R}_2$	»	7.1	7.4	-	-
$\bar{S}_3$	»	-	-	9.6	0.5
$\bar{R}_3$	»	-	-	9.6	7.4

Model II. Here we have: $D + R_1 = S_1 + E_1$, and $E_1 = E$, expressing quasi-stationarity in the P- and U-pools; hence $R_1 = S_1 + E - D$. Furthermore, $R_3 + S_1 = S_3 + R_1$, which means that the Q-pool is in a quasi-stationary state, hence $R_3 = S_3 + E - D$.

Table IV contains the parameter formulae for the organism, both in nitrogen equilibrium and in negative disequilibrium. In Table V are rendered all the computations of parameters.

Olesen, Heilskov & Schønheyder (1954 were not able to decide on the basis experiments performed on normal perso which of the models in Fig. 1 gave the be expression of nitrogen interaction in th organism. Below the changes in paramet sizes which occur during immobilization a discussed according to both models.

Model I. It will be noticed that in o and the same subject the pools P, Q and are of the same order of magnitude in t

o experimental conditions. This does not ntradict our postulate of quasi-stationarity r these homogeneous pools. We further d that in both subjects S_1, and hence per finition R_1, are of the same order of magni-de in immobilization experiments as well in normal experiments. Finally, examina-ns show that during immobilization there a pronounced decline in rate of synthesis $_2$) to the V-pool, while the rate of break-wn (R_2) from this pool seems unchanged m the normal state. As V is a blind pool, are not able to compute its size.

Model II. Table V shows in both subjects narked reduction in the size of the Q-pool ring immobilization. S_3 is clearly dimi-shed in *ES* and halved in *RB*. R_3 is almost changed. The reduction in the size of the -pool during immobilization is so obvious, *ES* 141 g and in *RB* 126 g of nitrogen the latter case related to the average of two normal experiments), that it seems probable that this change should only have en place in the days preceding the immo-ization experiments with glycine-N^{15}, and t the change should not be maintained o during the continued immobilization riod. If that is so then quasi-stationarity the Q-pool is not realized and there is no is for the parameter calculations. The duction in the size of the Q-pool is, more-er, so great that it could not be explained the total loss of nitrogen, *prior* to the mobilization experiment with glycine-N^{15}, consequence of the negative nitrogen ance (about 25 g in *ES* and about 22 g *RB*).

A reduction of the Q-pool due to a nitro-shift from the Q-pool to the V-pool in interval between the effectuation of the immobilized state and the following administration of N^{15}-glycine seems to us to be very unlikely. The conception of Model II as the way in which the organism treats nitrogen, and the principles which formed the basis for the evaluation of the parameters according to this model, are thus not confirmed by our experiments during immobilization. For this reason we feel inclined to leave out Model II from our considerations.

The question as to the homogeneity of the different pools has previously been discussed in details (Olesen, Heilskov & Schønheyder, 1954) and will not be further mentioned here.

The authors are aware of the possibility that the postulate of quasi-stationarity in the pools P, Q and U is less likely to be realized in the case of persons in negative nitrogen balance than in cases where there is nitrogen equilibrium, because, during the period in which the immobilization experiment with glycine-N^{15} is carried out, the amount of nitrogen in urine per day is lessened. The deviations from the average values of E during the experimental period are, however, at the most 9.4 per cent (Table II). It follows that all the parameters determined presumably are subject to similar fluctuations.

The method followed in the calculations of parameters according to Models I and II has the drawback that the considerations made involve that the negative nitrogen balance can only be produced at the cost of the V-pool.

The authors are unable to explain why the negative nitrogen balance goes toward a maximum and then shows a pronounced tendency to decrease.

SUMMARY

Negative nitrogen balance was produced in three normal subjects by immobilization in bed. The urinary excretion of N^{15} after administration of N^{15}-labelled glycine per os was checked in the same persons, partly in normal and partly in immobilized conditions. A much faster urinary excretion of N^{15} was found during negative nitrogen balance than during nitrogen equilibrium. The subjects were fed exactly the same diet under the different experimental conditions.

The kinetics of excretion of N^{15} were analyzed mathematically with reference to two different models for nitrogen interaction in the organism. By means of the empirically determined constants in the equations which render the excretion of N^{15}, the pool sizes and rates of flows were determined for the experimental subjects studied under normal conditions and during immobilization. These calculations were carried out according to Model I as well as to Model II.

The immobilization values obtained ac cording to Model II make it very unlikel that this model is a true representation c nitrogen interaction in the organism.

The values obtained for the different para meters according to Model I seem to indicat that the mechanism for the negative nitroge balance during immobilization is primaril based upon a reduced synthesis of tissu proteins while the rate of breakdown o these proteins remains unchanged.

ACKNOWLEDGMENTS

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REABSORPTION FROM URINE IN COLON

EXPERIMENTAL STUDIES ON SOME PATHO-PHYSIOLOGICAL CONDITIONS FOLLOWING URETEROCOLIC ANASTOMOSES[1,2]

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The first operation by which urine deliberately was led into the colon was performed by John Simon (1852). He planned operation on the basis of a knowledge the existence of cloacae in birds and on experience that humans who had been mitted to rectovesical lithotomy had maintained continence.

An unsuccessful ureterocolic anastomosis ds to very striking and serious complications. It is therefore quite understandable t for a long period the question of technily perfecting the operation almost entirely racted the attention from the problem of patho-physiological consequences of the esence of urine in the intestine.

Some passages written by Thomas Smith 1879 bear evidence that he at least was are of this problem, and in fact they ine quite well some of its major aspects. wrote, "It was less certain what would the effect on the colon of a constant flow urine over its mucous membrane, nor ld one feel sure that trouble might not se from absorption of urine by the intinal mucous membrane", and later "In connection with this subject, it may be worth considering how far the function of the kidney might be influenced by the introduction of intestinal gas to its pelvis".

The first analysis of the colonic excretion from patients with ureterocolic anastomoses was apparently published by Graser (1909). The urine mixed with faeces was collected in 24-hour samples from two children operated on, and the following observations were made, 1) the 24-hour volumes from the colon exceeded the diuresis of normal children of corresponding age, 2) nitrogen concentration was lower than that in the urine of the controls, though hardly lower than was accountable for by the increased volume, 3) the reaction of the colonic excretion was alkaline, and 4) unmixed urine, collected by rectal tube on a few occasions, had almost the same nitrogen concentration as that of the controls.

The results of this study thus suggested that an alteration of the composition of the urine actually occurs during its stay in the colon, but it was not until recently that the questions formulated by Thomas Smith were made a subject for more thorough investigation.

The interest was evoked by the statement (Ferris & Odel, 1950) that a particular type of electrolyte imbalance frequently occurs in

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patients with ureterocolic anastomoses, viz. "hyperchloremic acidosis", characterized by supranormal plasma chloride and subnormal plasma bicarbonate concentrations, often associated with an elevation of blood urea. Boyd had in 1931 made a similar observation but only in a single case.

PREVIOUS INVESTIGATIONS

Previous studies have already furnished some suggestive evidence that the contact of the urine with the mucous membrane of the colon may be an important causal factor in the development of hyperchloremic acidosis. Among the clinical observations attention should be called to the fact that patients with cutaneous ureterostomies do not suffer from hyperchloremia (Doroshow 1951, Pers 1952), and that a high incidence of diarrhoea has been observed among the patients who showed the most constant acidosis following ureterocolic anastomosis. Ferris & Odel (1950) stated further that some improvement may be attained by constant lavage of the colon with water.

In experiments Boyce & Vest (1952) have shown that ammonium chloride is readily absorbed from aqueous solutions in the colon of dogs and that the absorption brings about typical hyperchloremic acidosis as well as a rise in blood urea.

After all, the significance of urinary contact with the intestinal mucous membrane for the development of hyperchloremic acidosis in patients with ureterocolic anastomoses cannot be assessed by a simple analysis of the electrolyte alterations in plasma, because the influence of a possible renal insufficiency cannot be excluded. Studies on the absorption from simple solutions of electrolytes do not solve the problem either, becaus it is not permissible to transfer these experi mental results to such a complex system a urine.

Analyses have also been made of urin before and after its retention in the colon Parsons, Powell & Pyrak (1952) point ou that in cases where only one ureter has bee transferred to the colon, the urine from th bladder is acid and contains more chlorid and NPN than the alkaline excretion simul taneously collected from the colon. It ha further been shown that when urine con taining labelled chloride and sodium was in troduced into the rectum there occurred greater percentile loss of labelled chlorid than of labelled sodium (Parsons, Pyrak Powell, Reed & Spiers 1952). Such isotop experiments give no information, however on the net absorption because the extent o exchange of ions is unknown.

The net reabsorption from urine intro duced into the isolated distal colon in tw patients on whom a colostomy had bee made, was studied by Annis & Alexande (1952). In the course of three hours th concentrations of potassium and uric aci remained fairly constant whereas a consider able decrease occurred in the chloride con centration exceeding that of the sodium con centration. Accordingly there is reason t assume that urinary electrolytes are re absorbed through the colonic mucosa.

Lapides (1951) found that a constant in stillation of a subject's own urine into th large bowel brought about hyperchloremi acidosis and an elevation of blood urea i patients with impaired renal functio whereas this procedure was without effe on the electrolyte balance and blood ure

el in normal subjects. By these experi-
nts it seems demonstrated that impaired
al function predisposes to hyperchloremic
losis, but they do not allow us to conclude
the hyperchloremic acidosis is due to a
al inability to excrete chloride, hydrogen
equivalents, and urea at a suitable rate.
possibility also exists that the urine
duced by the impaired kidneys was of
a nature as to favor the intestinal
sorption of chloride, hydrogen ion equi-
nts, and urea.

he aim of the present experiments has
to study the net reabsorption by the
n of chloride, hydrogen ions, urea, etc.,
n urine specimens of different composi-
s. Special attention will be given to the
stion of which type of urine involves the
test risk of causing hyperchloremic aci-
s and elevation of blood urea in patients
ureterocolic anastomoses.

METHODS

Animal experimental methods.

o achieve a quantitative determination of the
reabsorption, a well-defined section of the colon
to be used, into which a definite amount of
of known composition could be introduced
from which the remainder could be withdrawn
titatively after a suitable experimental period.
o make the experimental conditions as uniform
ossible it was decided to use the same animal
all the experiments, and a dog was operated
the following way:

he intestine was divided just oral to the
aecal junction and at the middle of the sigmoid.
caecum with the ileocaecal junction was re-
ed and the isolated — about 20 cm long —
ent of colon was closed at both ends around
collar cannulae, which were passed through
lateral abdominal walls, one on each side.
lly the ileum and the sigmoid were joined
. 1).

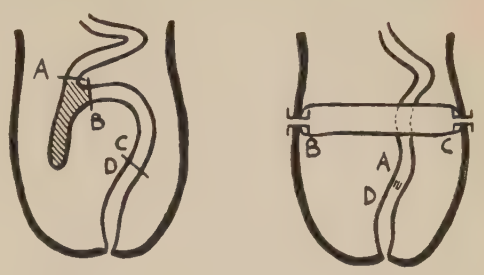


Fig. 1. Method used to procure a permanent isolated segment of the colon in a dog.

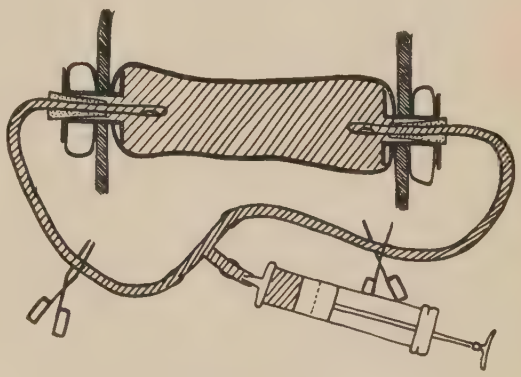


Fig. 2. Arrangement employed during the experiments.

After a period of convalescence the dog did well. The faeces were pasty, and during the next 12 experimental months the dog displayed no signs of dehydration or nutritional deficiency. The dog was trained and the experiments could be performed without anesthesia or sedatives.

The collar cannulae could be made close-fitting by means of a tight cotton packing which pressed the inner collar of the cannula against the mucous membrane during the experiments. Short catheters were wedged into the cannulae, and the system was closed by connecting the catheters. The isolated segment could hold maximally 120 ml. By minor distension of the intestine (and in the absence of too much mucus) the fluid could be seen to circulate by peristalsis. During the experiments mixing of the content was further promoted by pumping the fluid around every 20 minutes with a syringe (Fig. 2).

Some mucus accumulated in the intestinal segment, which made washing before the experiment desirable. The following procedure was employed to avoid retention of wash-fluid: 150 ml of urine were measured out and about 90 ml used for irrigations. After exactly 90 ml had been evacuated, the rest was introduced, and 10 ml of this were withdrawn again, constituting the first sample, while 50 ml (plus a small amount, corresponding to the washed-out mucus) remained in the intestine. In this way differences of any size were avoided in the initial volume due to varying retention of wash-fluid, and withdrawal of the initial sample from the intestine prevented the admixture of mucus from influencing the experimental results.

Human urine was used, partly urine specimens of different concentrations from normal subjects, and partly specimens from patients with postoperative electrolyte disturbances (Nos. 13, 16 and 17).

Patients with ureterocolic anastomoses usually void at intervals of about four hours; hence an experimental period of two hours was chosen, corresponding to the average time that the urine is retained in the large bowel by the patients.

At the end of the experiment a sample was first withdrawn anaerobically for analysis before the remainder was removed as completely as possible by passing air through the segment. In order to determine the amount of urine now remaining in the segment, the latter was washed with enough water to allow the withdrawal of a total of 100 ml. By urea determination on this wash-water and on the undiluted final urine the amount of urine contained in the wash-water could the calculated (4—8 ml), and this amount was added to the already measured quantity.

Analytical methods

Specific gravity of the urine was measured with an ordinary hydrometer.

pH was measured on a pH-meter with glass electrode and calomel reference electrode. The measurements were made at room temperature.

Titratable acid was determined in connection with the pH measurement by titrating a known amount of urine with NaOH to pH 7.4 at room temperature.

Bicarbonate. Total carbon dioxide was determined by van Slyke's method (1917), and the bicarbonate content of the urine was calculated from the existing pH, according to the Henderson-Hasselbach equation ($pK = 6.1$).

Urea and *ammonia* were determined by Conway's microdiffusion method (1933) with and without addition of urease. The preformed ammonia was subtracted in the calculation of the urea.

Chloride was determined by electrometric titration with $AgNO_3$.

Phosphate was determined colorimetrically, by a modification of Fiske & Subarrow's method (1923). Milligrams phosphorus was converted into mE of phosphate at pH 7.4.

Sodium and *potassium* were determined by flame photometry (Berry, Chappel & Barnes 1946) with internal standard (lithium) and under control of the influence of potassium on the sodium analysis.

The urine was stored under toluene at 4° C.

The determinations by flame photometry were based on duplicate readings. As to the remaining analyses, the whole procedure was carried out in duplicate.

Methods of calculation

During the formation of urine, hydrogen ions may be removed from the organism or added to it dependent on the composition of the urine. The titratable acid of the urine as well as its ammonium content represent a removal of hydrogen ions from the organism. The excretion of bicarbonate in the urine, on the other hand, represents an addition of hydrogen ions to the organism, (since it causes a fall in the bicarbonate concentration of the body fluids, and consequently a dissociation of carbonic acid into hydrogen ions and bicarbonate).

The total number of hydrogen ion equivalents removed from the organism in a certain amount of urine may therefore to a good approximation be calculated as [titratable acid $+ NH_4^+ \div HCO_3^-$].

Correspondingly determinations of this quantity in the urine introduced in the colon and in that remaining at the end of the experimental period allow a calculation of the total number of hydrogen ion equivalents returned to the organism from the urine.

n cases of ureterocolic anastomoses it is a ter of special interest to determine the *per-tages* (of hydrogen ion equivalents, chloride,) which are reabsorbed in the intestine, since this cates the degree to which the renal excretion is dered inefficient.

y calculating the percentile net absorption for oride, hydrogen ion equivalents and urea we are hermore able to compare the chances of these stances of being reabsorbed from different urine cimens, and possibly to decide whether the centile net absorption of the individual sub-ces is particularly great or particularly small n certain types of urine.

RESULTS AND DISCUSSION

The results of the analyses are presented ow in tabular form. The net absorption hydrogen ion equivalents was calculated n the variation of the expression [titrat-e acid + $NH_4^+ \div HCO_3^-$] during the y of the urine in the colon within the erimental period. In addition, the varia-s are indicated for volume, pH, the most portant anions and cations, as well as a.

Volume

The tables show that the volume of the estinal contents increased when urine cimens with a specific gravity over 1019 re introduced, whereas it decreased or nained unchanged after introduction of ne with a lower specific gravity.

The net water movement follows the notic gradient in direction and rate isscher, Fetcher, Carr, Gregor, Bushey Barker 1944). The experiments of these iters, where sodium chloride solutions re used in the small intestine of dog, did show an absolutely linear relationship ween the net water movement and the notic activity in the intestinal content.

Accordingly, in the present study the final volume has been used only as a *relative* measure of the total osmotic activity in the urine specimens employed.

Such a classification of the urine specimens on the bases of the volumes after two hours seems justifiable, since in other experiments were the changes in volume were followed at short intervals, it has been shown that a shift in the direction of the fluid movement never occurred within the first two hours.

The experimental period was, as stated, always two hours irrespective of the concentrations of the urine specimens. The objection might *a priori* be raised that concentrated urine, formed as a result of a low rate of urine flow, would obtain a long period of contact before a need for voiding would arise, whereas urine of low concentration, due to a high rate of urine flow, would leave the colon after a shorter period of contact. However, since the volume of a concentrated urine specimen increases during its stay in the intestine, while that of a dilute decreases, it may be justified to assume that all types of urine have approximately the same time of contact in the bowel.

In patients with ureterocolic anastomoses the postrenal changes in urine volume will reduce their chances of regulating the water excretion by altering the rate of urine flow.

Chloride

When the chloride concentration in the urine introduced was below that of plasma, some 75 to 100 per cent of the urine chloride was reabsorbed. At higher chloride concentrations a lower percentage of net reabsorption was generally observed (Fig. 3).

Table I. *Changes in hypertonic urine specimens.*

No.		Spec. grav.	Vol. ml	pH	Titr. acid mE	NH_4^+ mE	HCO_3^- mE	H^+ mE	Cl^- mE	PO_4^{--} mE	Na^+ mE	K^+ mE	Urea mg
1.	Before	1025	50	5.67	1.58	2.87	0.04	4.41	6.95	1.91	5.15	5.32	1700
	After		122	7.24	0.15	2.07	2.90	−0.68	4.87	1.74	8.42	5.37	1495
	Diff. △		-	-	1.43	0.80	−2.86	5.09	2.08	0.17	−3.27	−0.05	205
	%		-	-	-	-	-	115.1	29.9	-	-	-	12
4.		1028	50	5.27	2.96	3.95	0.01	6.90	7.45	3.81	6.60	5.20	1147
			85	6.50	1.26	2.34	0.57	3.03	2.21	3.81	5.35	5.52	1040
			-	-	1.70	1.61	−0.56	3.87	5.24	0.00	1.25	−0.32	107
			-	-	-	-	-	56.1	70.2	-	-	-	9
5.		1024	50	5.08	2.10	2.58	0.01	4.67	10.45	2.70	6.05	5.30	1076
			78	6.68	0.70	1.72	0.79	1.53	4.45	2.94	3.85	3.67	679
			-	-	1.40	0.86	−0.78	3.04	6.00	−0.24	2.20	1.63	397
			-	-	-	-	-	65.0	57.4	-	-	-	37
6.		1026	50	5.61	1.20	2.68	0.04	3.84	7.50	1.78	4.30	4.05	815
			76	6.71	0.45	1.05	0.67	0 83	1.98	1.91	3.19	2.77	709
			-	-	0.75	1.63	−0.63	3.01	5.52	−0.13	1.11	1.28	106
			-	-	-	-	-	78.4	73.6	-	-	-	13
8.		1023	50	5.18	1.60	3.05	0.02	4.63	8.75	1.22	5.80	4.45	1068
			99	6.80	0.60	1.93	1.27	1.26	3.27	1.45	4.45	2.48	932
			-	-	1.00	1.12	−1.25	3.37	5.48	−0.23	1.35	1.97	136
			-	-	-	-	-	72.6	62.6	-	-	-	12
11.		1021	50	5.80	3.30	4.17	0.05	7.42	5.40	5.34	4.40	5.50	1220
			90	6.65	1.80	3.67	0.86	4.61	1.80	5.41	4.50	4.86	1170
			-	-	1.50	0.50	−0.81	2.71	3.60	−0.07	−0.10	0.64	50
			-	-	-	-	-	36.5	66.6	-		-	4
13.		1030	50	4.50	3.45	3.85	0.00	7.30	8.15	1.02	4.40	7.70	1034
			82	5.20	0.65	2.25	0.04	2.86	3.20	1.02	5.25	7.05	840
			-	-	2.80	1.60	−0.04	4.44	4.95	0.00	−0.85	0.65	194
			-	-	-	-	-	60.6	39.3	-	-	-	18
15.		1019	50	5.68	1.10	1.77	0.04	2.83	5.75	1.54	5.45	3.10	940
			86	7.03	0.29	1.00	1.47	−0.18	2.15	1.68	3.91	4.08	810
			-	-	0.81	0.77	−1.43	3.01	3.60	−0.14	1.54	−0.98	130
			-	-	-	-	-	106.1	62.6	-	-	-	13
16.		1024	50	5.81	4.90	3.03	0.04	7.89	1.20	3.14	0.38	2.02	1762
			88	6.55	0.97	1.49	0.55	1.91	0.27	2.23	1.80	1.91	1330
			-	-	3.93	1.54	0.51	5.98	0.93	0.91	−1.42	0.11	432
			-	-	-	-	-	74.6	77.5	-	-	-	24

Since high chloride concentrations were usually associated with a high total osmotic activity in the urine specimens, the percentage of net reabsorption was found to be low at high total osmotic activity (Fig. 4).

By comparing the two diagrams and especially noting, in Fig. 3, the slight re absorption of chloride in experiment No. (low chloride concentration — high tota osmotic activity) in proportion to that i experiment No. 14 (high chloride concen tration — low total osmotic activity), we ge

Table II. *Changes in hypo- or isotonic urine specimens.*

	Spec. grav.	Vol. ml	pH	Titr. acid mE	NH_4^+ mE	HCO_3^- mE	H^+ mE	Cl^- mE	PO_4^{--} mE	Na^+ mE	K^+ mE	Urea mg
Before	1014	50	5.93	0.75	1.09	0.05	1.79	3.30	1.13	2.50	2.65	733
After		50	7.14	0.15	0.37	0.67	−0.15	0.12	1.22	0.85	2.50	514
Diff. △		-	-	0.60	0.72	−0.62	1.94	3.18	−0.09	1.65	0.15	219
%		-	-	-	-	-	108.1	96.4	-	-	-	29.8
	1006	50	6.34	0.54	0.61	0.11	1.04	4.95	1.10	3.90	1.50	418
		18	6.89	0.27	0.56	0.14	0.69	0.06	1.03	0.40	0.92	150
		-	-	0.27	0.05	−0.03	0.35	4.89	0.07	3.50	0.58	268
		-	-	-	-	-	33.6	98.6	-	-	-	64.0
	1010	50	5.53	0.80	2.01	0.03	2.76	4.75	0.95	3.55	2.62	575
		51	7.00	0.12	0.86	0.65	0.33	0.10	0.97	1.04	1.79	373
		-	-	0.68	1.15	−0.62	2.45	4.65	−0 02	2.51	0.83	202
		-	-	-	-	-	88.6	97.9	-	-	-	35.0
	1008	50	5.27	0.45	1.45	0.02	1.88	2.65	0.51	3.40	1.00	442
		10	6.97	0.04	1.15	0.28	0.91	0.25	0.11	1.40	0.60	208
		-	-	0.41	0.30	−0.26	0.97	2.40	0.40	2.00	0.40	234
		-	-	-	-	-	51.6	90.6	-	-	-	53.0
	1006	50	7.19	0 04	0.61	0.93	−0.28	3.75	0.47	4.80	0.85	382
		17	7.11	0.04	0.38	0.22	0.20	0.09	0.39	0.55	0.53	160
		-	-	0.00	0.23	0.71	−0.48	3 66	0.08	4.25	0.32	222
		-	-	-	-	-	−171.0	97.6	-	-	-	58.0
	1010	50	6.21	0.23	1.75	0.13	1.85	4.25	0.34	3.87	2.07	617
		42	7.30	0.01	1.53	0.69	0.85	0.12	0.32	1.00	1.49	428
		-	-	0.22	0.22	−0.56	1.00	4.13	0.02	2.87	0.58	189
		-	-	-	-	-	54.0	97.0	-	-	-	30.6
	1016	50	7.09	0.20	0.83	1.32	−0.29	12.25	1.51	12.30	4.27	601
		48	7 30	0.10	0.22	1.23	−0.91	2.21	1.01	3.79	2.50	376
		-	-	0.10	0.61	0.09	0.62	10.04	0.50	8.51	1.77	225
		-	-	-	-	-	−21.4	82.1	-	-	-	37.4
	1018	50	5.82	0.60	3.60	0.05	4.15	2.68	0.72	0.24	0.97	193
		35	6.25	0.42	0.85	0.15	1.12	0.04	0.83	1.00	1.22	140
		-	-	0.18	2.75	−0.10	3.03	2.64	−0.11	−0,76	−0.25	53
		-	-	-	-	-	72.9	98.5	-	-	-	27.5

impression that it is the osmotic activity he urine rather than its chloride con- ration which determines the percentage reabsorption of chloride.

The absorption of chloride from NaCl tions introduced in isolated loops of both small and large intestine has repeatedly studied. From hypo- and isotonic solu- s the percentage of net absorption was practically constant while in cases of hypertonic solutions a lower percentage of net absorption occurred the higher the NaCl concentration in the solution (Haidenhain 1893, Goldschmidt & Dayton 1919, Rabinowitch 1927, Budolfsen 1952).

Since osmotic activity varies in direct proportion to the NaCl concentrations in such solutions, these observations are in

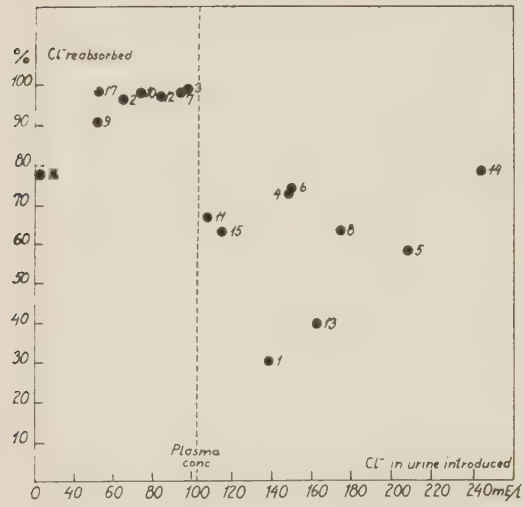


Fig. 3. Percentile net reabsorption of chloride in relation to initial concentration in the urine specimens.

accordance with the above mentioned results from experiments with urine specimens (Fig. 4).

Budolfsen (1952) has studied the absorption of NaCl from both the small and large intestines of rats. He found that the fall in the percentage of net absorption, with rising NaCl concentration in the intestine, was greater for sodium than for chloride.

In the interpretation of this finding it must be recalled that the net absorption is the resultant of an absorption process (gut → blood) and a secretion (blood → gut). Though differences in the rates of the absorption of sodium and chloride cannot be excluded, an increasing production of a secretion with a higher concentration of sodium than of chloride seems a more likely explanation.

According to calculations based on isotope experiments (Visscher, Fetcher, Carr, Gregor, Bushey & Barker 1944) with NaCl solutions in loops of the small intestine in dog the composition of the secreted fluid is fairl constant, and the "virtual" concentration sodium higher than that of chloride. Bee Johnston & Wilson (1926) likewise found constant composition of the spontaneou secretion of the colon with a higher concen tration of sodium (153 mE/l) than chloride (85 mE/l). The reaction was alka line, and the bicarbonate concentration hig (86 mE/l).

In the present experiments the net absorp tion of chloride has also been found to excee that of sodium, and furthermore the di ference was observed to increase with risin osmotic activity in the urine specimen (Fig. 5). In several experiments (Nos. 11, 13, 16 and 17) the sodium contents withi the intestinal segment actually did *increas* during the experimental period. Thus a least in these cases sodium must have bee secreted.

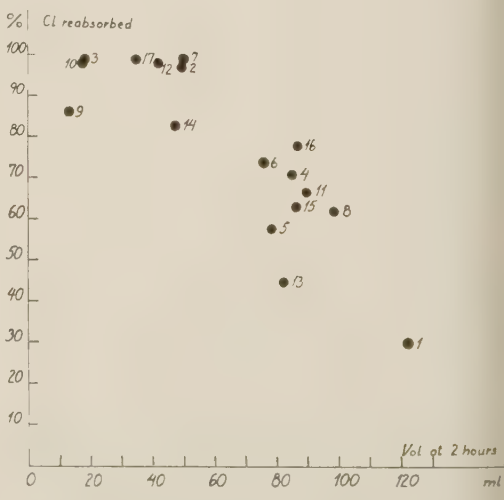
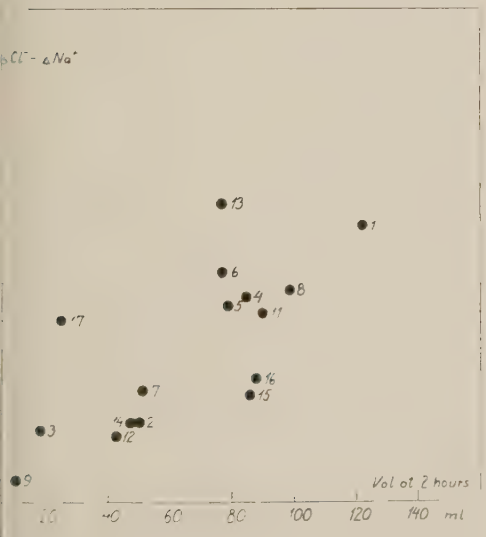


Fig. 4. Percentile net reabsorption of chloride relation to final volume.

Production of a secretion composed like above mentioned spontaneous secretion, uld, in fact, reveal itself as a reduction he net reabsorption of chloride and even re in that of sodium.

The increasing disparity between the net bsorptions of chloride and sodium when urine becomes more hypertonic, could be ounted for by assuming an increase in rate of formation of such a secretion.

We cannot say whether the increased otic activity itself is the factor responsible the fall in percentage of net reabsorption. nerally, the hypertonic urine specimens uld also be those which had removed the atest number of hydrogen ion equivalents n the organism, and the "acidity" or er factors may as well be conceived to e exerted a stimulating influence on the retion.



5. Differences in net reabsorption of chloride nd sodium from urine specimens in relation to final volume.

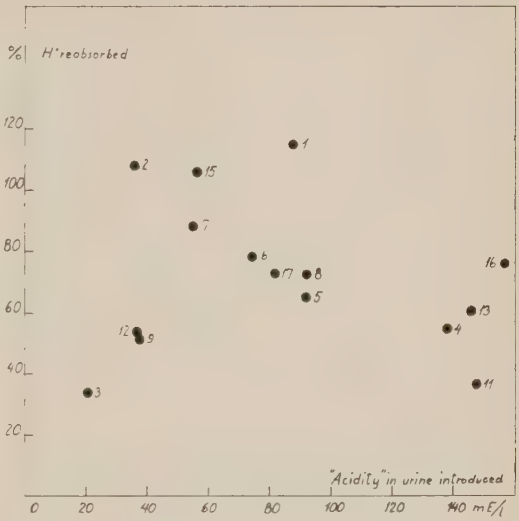


Fig. 6. Percentile net reabsorption of hydrogen ion equivalents in relation to the amount removed from the body by the urine specimen employed.

We may conclude from the above findings that the formation of a fairly dilute urine offers the best opportunity for the intestinal reabsorption of chloride and thus for chloride retention in patients with ureterocolic anastomoses.

Hydrogen ion equivalents

The largest net reabsorption of hydrogen ion equivalents occurred from such urine specimens as would have removed moderate amounts of hydrogen ion equivalents from the organism (Fig. 6). Absorptions exceeding 100 per cent indicate that more hydrogen ion equivalents were added to the organism than the urine sample concerned had removed by its formation. The experiments with alkaline urine specimens (Nos. 10 and 14) are not included.

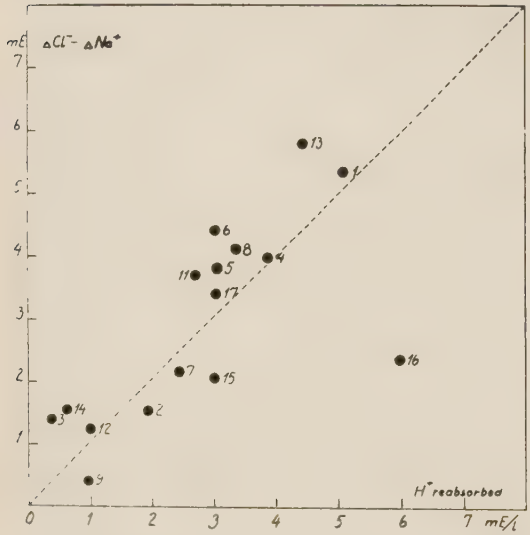


Fig. 7. Differences in net reabsorption of chloride and sodium from urine specimens in relation to net reabsorption of hydrogen ion equivalents.

The hydrogen ion equivalents added to the organism were, as previously explained, calculated from the change in [titratable acid $+ NH_4^+ \div HCO_3^-$] in the urine during the experiment. According to Gamble, Ross & Tisdall (1923) the titratable acid $+ NH_4^+ \div HCO_3^-$ may also be expressed as fixed anions ÷ fixed cations of the urine. Consequently the hydrogen ion equivalents returned to the organism from the urine may also be calculated from the change in: $[Cl^- + PO^{--} +$ organic acid $+ SO_4^{--}] \div [Na^+ + K^+ + Ca^+ + Mg^+]$ during the experiment.

Only small quantities of potassium and phosphates were reabsorbed from the urine specimens. Provided Ca, Mg, SO_4 and organic acids undergo no appreciable quantitative changes either, it follows that the net reabsorption of hydrogen ion equivalents must be approximately expressed by the change in $[Cl^- \div Na^+]$ which occurs during the experiment, $[\triangle Cl^- \div \triangle Na^+]$. As apparent from Fig. 7, the net reabsorption of hydrogen ion equivalents was, in fact, determined with good approximation from $[\triangle Cl^- \div \triangle Na^+]$.

This parallelism is comprehensible, because $[\triangle Cl^- \div \triangle Na^+]$ must depend partly on the extent to which Na^+ is secreted without Cl^- *i. e.* either with HCO_3^- or by a direct exchange between Na^+ from the organism and H^+ from the urine, and partly on the extent to which Cl^- is reabsorbed without Na^+, *i. e.* with NH_4^+ of H^+ from the urine.

The addition of hydrogen ion equivalents to the body from the urine in the colon should thus depend on the following mechanisms:

1) Secretion of HCO_3^- with fixed cation (Na^+).

2) Reabsorption of H^+, either together with anion (Cl^-) or by exchange with fixed cation (Na^+).

3) Reabsorption of NH_4^+ with anion (Cl^-).

The amount of bicarbonate secreted is presumably determined by the rate of intestinal secretion. As, however, the urine already contains buffer systems, part of the secreted bicarbonate will, provided the pH of the urine is lower than that of the secretion, react with hydrogen ions originating from these buffers. The titratable acid is thereby reduced. Part of the bicarbonate secretion is therefore measured as a fall in the content of titratable acid in the urine and not as a rise of the bicarbonate concentration.

Conversely, a possible direct H^+ absorption leads not only to a fall in the content of titratable acid, but owing to the rise in

H, also to an increase in the bicarbonate ncentration in the urine, as the carbon oxide tension can be regarded as constant d in equilibrium with the blood.

The possibility exists that a certain amount NH_3 was formed during the experiment om the urea contained in the urine. How-er, such an NH_3 formation represents no urce of error in the determination of the dition of hydrogen ion equivalents to the ganism. NH_3 will, it is true, take up H^+ the intestine with formation of NH_4^+, t the rise in NH_4^+ will be made up for a corresponding change in titratable acid d bicarbonate and [titratable acid + NH_4^+ HCO_3^-] is therefore not influenced. On other hand, NH_3 formation, of course, l make it impossible to assess how great hare absorption of NH_4 has in the addi-n of hydrogen ion equivalents to the ganism.

Finally, it must be mentioned that NH_4^+ rmation in the urine during its retention the intestine may be conceived to alter conditions for the addition of hydrogen s to the organism, (*e. g.* NH_4^+ ions may ssibly be reabsorbed more readily than drogen ions in the existing concentra-ns).

Thus the three components upon which calculation of the total addition of hydro-n ions to the organism is based are so imately interrelated that an analysis of ir individual shares is impossible.

As stated above, there is, however, reason suppose that considerable amounts of a retion rich in bicarbonate may be pro-ced in the colon and it appears from the perimental results that the total osmotic ivity bears some relation to the addition

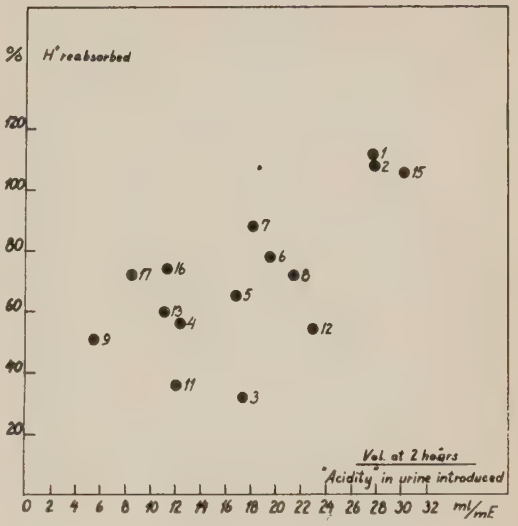


Fig. 8. Percentile net reabsorption of hydrogen ion equivalents in relation to the ratio between final volume and the amount of hydrogen ion equivalents removed from the body by the urine specimen employed.

of hydrogen ion equivalents to the body. In Fig. 8 the percentage of net reabsorption of hydrogen ions is plotted against the total osmotic activity (volume at 2 hours) divided by the "acidity", and it appears that the percentile addition of hydrogen ions to the body tends to be high when the total osmotic activity is high in proportion to the "acidity" of the urine specimen.

This relationship is probably due to an increased formation of a secretion (rich in bicarbonate and sodium, less so in chloride) which also, as suggested above, may explain the reduction in net reabsorption of chloride and sodium.

Patients with ureterocolic anastomoses should thus be most liable to retain hydrogen ion equivalents when the amount that a certain quantity of urine removes from the

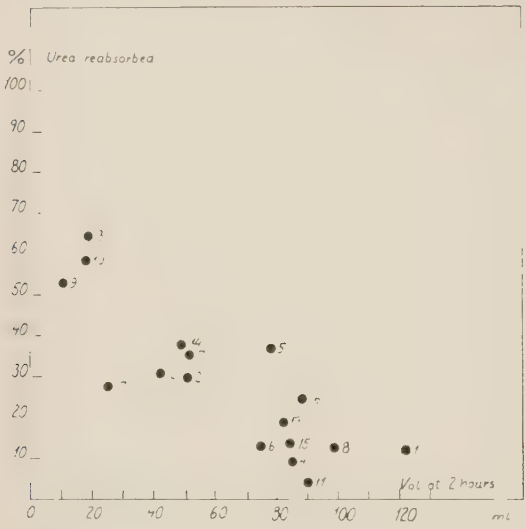


Fig. 9. Percentile net reabsorption of urea in relation to final volume.

organism is moderate (Fig. 6), and especially if it is small in proportion to the total osmotic activity of the urine (Fig. 8), *e. g.* if the rate of urine flow is fairly high and the osmotic activity still considerable.

Urea

Urea has a considerable share in the osmotic activity of the urine. The final volume also proved to follow fairly closely the urea concentration of the initial urine.

The absolute quantity of net reabsorbed urea was almost constant, independent of the urea concentration. The percentile net reabsorption, accordingly, was the lowest at high concentration of urea or large final volume (Fig. 9).

Conversion of urea to NH_3 might represent a source of error. In 10 experiments a sample of urine used for the washing of the intestinal segment was therefore incubated at 37° for 2 hours. This control showed that the changes in NH_3 and urea concentrations amounted to a few per cent only of their initial values.

Urea is a highly diffusible anelectrolyte. We should therefore *a priori* expect the net reabsorption to have risen with the gradient. As however, the hypertonic urine proved to increase in volume during the reabsorption, there must, simultaneously with the diffusion of urea out of the intestine, have occurred an osmotically determined fluid movement in the opposite direction. This mass movement must have tended to depress the diffusion.

The rates of penetration of fluid into the intestine and of urea out of the intestine were determined by the osmotic activity and the urea gradient respectively, which both depended on the urea concentration in the urine introduced. Hence, it seems likely that it was the inhibition by this greater fluid movement into the intestine that made up for the expected urea diffusion out of the intestine due to the presence of a high gradient in cases of hypertonic urine. Using hypotonic urine specimens, fluid was seen to move in the opposite direction and thus to favour the diffusion of urea.

Thus, retention of urea is likely to occur in patients with ureterocolic anastomoses if the urine is hypotonic.

CONCLUSION

The experimental results indicate that, in patients with ureterocolic anastomoses, the postrenal changes of urine volume make the renal regulation of the water excretion inefficient in such a way that the water balance depends in the first instance on the intake of fluid.

Evidence has previously been found to gest a higher net absorption rate for bride than for sodium, when reabsorbed m urine in the colon. This finding has n confirmed, and it may be added that difference between the amounts re-orbed within two hours increases with ng concentration of the urine. The ium content of the urine may even in-ase, indicating production of a sodium-taining secretion.

t seems reasonable to explain this fact hat highly concentrated urine stimulates production of an intestinal secretion con-ing much sodium and bicarbonate, and chloride.

The percentile net absorption of urinary stituents by the colon is a significant or in the conditions following uretero-c anastomoses. It is therefore a matter importance that large differences have n demonstrated in the percentile net re-orption rates, dependent on the composi-n of the urine, especially its concentration. The largest percentile net reabsorption of bride and urea occurs from the least con-trated urine specimens and, as regards drogen ion equivalents, the greatest risk retention is present when the amount moved from the body by formation of a ain quantity of urine is moderate, and ecially if it is small in proportion to the al osmotic activity of the urine. The nges in $[\triangle Cl^- \div \triangle Na^+]$ are a fairly d measure of the addition of hydrogen equivalents to the organism.

t has previously been shown that instilla-into the colon of a subject's own urine to hyperchloremic acidosis when the al function was impaired. According to the results of the present study, this is, at least in part, accountable for by the fact that patients with impaired renal function may produce urine that, due to low concentration and moderate "acidity", favors reabsorption by the colon.

The considerable percentile net reabsorption by the colon of chloride and urea, when the urine is not concentrated to hypertonicity by the kidneys, and of hydrogen ion equivalents when the "acidity" is moderate, thus seem to be factors essential for the development of the hyperchloremic acidosis and the elevation of blood urea following ureterocolic anastomoses.

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TUDIES ON THE EFFICIENCY OF VITAMIN K_1 IN SMALL DOSES AS ANTIDOTE AGAINST ANTICOAGULANTS OF THE DICOUMAROL TYPE*

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The increasing use of dicoumarol and its alogues in anticoagulant therapy has greatly hanced the risk of hemorrhagic complica-ns through overdosage. In the series blished by Wright, Marple & Beck (1948) morrhagic complications occurred in 7 r cent of the cases, and according to Russek Zohman (1952) 122 fatal cases due to ticoagulant treatment had occurred in edical centers in the United States up to at time. From Denmark 2 unquestionable ses of death caused by dicoumarol treat-ent have been reported (Holten 1951, arrestrup Andersen 1953).

A number of investigators have shown at the hypoprothrombinemia induced by ese anticoagulants may be efficiently unteracted by large doses of vitamin K_1 vitamin K_1 oxide usually given intraven-sly as emulsions (Davidson & MacDonald 943, James, Johnson & Burnett 1951, Van allie, Geyer & Star 1951a, Watkin, van allie, Logan, Geyer, Davidson & Star 1951, ehbein, Jaretzki & Habif 1952, Koller, eliger & Ducker 1952, 1953, Guttas, Molo-y & Sise 1953, Brown & MacMillan 953).

In the hands of most workers menadione or its simpler derivatives had no or only slight effect against overdosage with dicoumarol and similar anticoagulants. (James, Bennett, Scheinberg & Butler 1949, Miller, Harvey & Finch 1950, Van Itallie, Geyer & Star 1951b, Barker, Hanson & Mann 1952, Douglas & Brown 1952, Van Buskirk 1953, Bannon, Owen & Barker, 1953).

By contrast, a marked effect of menadione and its simpler derivatives has been reported by Cromer & Barker (1944), Bjerkelund (1950) and Overman, Sorenson & Wright (1951) who gave menadione bisulfite intravenously. Recently Hilden & Munck (1953) found a similar effect of menadione sodium bisulfite given orally or intramuscularly, although with some variation from patient to patient.

Many authors recommend doses of 500—1000 mg of vitamin K_1, given intravenously as aqueous emulsions. Administering such large amounts introduces a risk of thrombosis when the prothrombin activity increases above the therapeutic interval. Furthermore, large doses may lead to a more or less pronounced resistance to dicoumarol therapy during a certain period.

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In the present study the effect of small doses of vitamin K_1 upon the hypoprothrombinemia induced by two long-acting anticoagulants is examined. A comparison with two of the simpler menadione derivatives (in large doses) has also been conducted.

MATERIALS AND METHODS

The following two anticoagulants were used: Dicoumarol (3,3'-methylene-bis-4-hydroxycoumarine), G 25766 (2-p-chlorphenyl-1,3-indanedione).[1] The vitamin K preparations were the following: A sterile transparent colloidal solution of vitamin K_1 in water (10 mg K_1 per ml), prepared by two of the authors (Dam & Søndergaard).[2]

Konakion, a colloidal aqueous solution of vitamin K_1 in ampoules (Trinkampullen) (10 mg K_1 in 0.2ml), furnished by F. Hoffmann-La Roche & Co., A. G., Basle. Menadione sodium bisulfite in tablets (Ph. Dan.) each containing 10 mg of the substance corresponding to 5.2 mg menadione. Menadione sodium bisulfite in sterile aqueous solution (Ph. Dan.) containing 5 mg of the substance in 1 ml, corresponding to 2.6 mg of menadione. Menadiol sodium sulfate (K-vimin, IDO) in sterile aqueous solution, containing 5 mg of the substance in 1 ml corresponding to 2.08 mg of menadione.

The method described by Owren & Aas (1951), as modified by Aas (1952) was used for determination of the combined effect of prothrombin and proconvertin (the so-called PP-method). The studies were performed on 40 persons over 60 years of age, none o whom showed signs of liver disease. Twenty two received dicoumarol, 18 G 25766. Th substances were given orally in doses suffi cient to depress the PP-concentration belo 10 per cent of the normal value. When thi level was reached the patients received vita min K as specified in Table I. Hemorrhage did not occur in any of the patients ever with PP-values as low as 3 per cent.

RESULTS AND DISCUSSION

The results are shown in Table I. Som of the typical cases are illustrated in Figure 1—5. It is seen that vitamin K_1 was th only substance tested which had a marke effect. Even in large doses (100 mg) th two simpler derivatives of meadione gave n or slight effect. Intravenous administratio of vitamin K_1 solution was the most efficien route. In this way it was possible to obtai an effect sufficient to eliminate the risk o serious hemorrhages within two hours, ever with doses of 10—20 mg. Oral ingestio also raises the PP-value, but it requires fiv to six hours to reach values equal to thos obtained after two hours by intravenous in jection.

Thus, our experiments confirm the supe riority of vitamin K_1 over the simpler mena dione derivatives. Further, they show that i is possible to elevate the PP-value to level usually considered outside the range i which serious hemorrhages may occur b means of small doses which do not result i complete normalisation, even after 24 hours The low efficiency of menadione etc. show that the beneficial effect obtained with vita min K_1 is unrelated to the fact that the ant

[1] This substance was obtained from J. R. Geigy A. G., Basle.

[2] The same preparation as used in studies in chicks and rats (Dam & Søndergaard 1953 a, b, Dam, Prange & Søndergaard, 1954).

Table I. *Influence of some forms of vitamin K administered in different ways on low prothrombin-proconvertin concentrations (PP-values) produced by anticoagulants of the dicoumarol type.*

Forms of vitamin K and mode of administration	Amount of vit. K administered mg	Anti-coagulant used*	Hours after administration of vitamin K				
			0	1	2	6	24
			PP-value in % of normal				
Vitamin K_1, intravenous	5	CPID	3	5	9	19	30
		CPID	9	11	13	24	41
		CPID	8	13	20	54	60
		D	9	12	15	27	24
		D	7	7	13	20	35
	10	CPID	5	10	14	52	62
		CPID	12	9	10	19	33
		D	10	12	17	32	43
		D	7	9	15	25	46
		D	6	-	16	-	32
		D	8	14	16	31	41
	20	CPID	6	12	14	28	41
		CPID	7	15	22	40	66
		D	7	20	20	20	37
		D	9	15	20	52	62
		D	7	30	46	44	63
Konakion, Roche, oral	5	CPID	7	9	10	18	32
		CPID	6	6	7	14	19
		CPID	10	12	12	25	38
		D	10	11	11	10	21
	10	CPID	12	12	11	26	50
		CPID	10	12	12	25	38
		D	9	10	11	13	27
		D	10	10	12	12	23
	20	CPID	17	20	21	36	55
		CPID	6	9	12	26	62
		D	9	9	11	15	44
		D	8	10	19	25	37
Menadione sodium bisulfite, intravenous	10	CPID	12	10	11	14	18
		CPID	14	12	13	16	18
	45	D	9	12	18	19	12
	100	CPID	7	7	7	8	10
		CPID	5	4	4	4	4
Menadiol sodium sulfate, intramuscular	50	D	4	3	3	6	6
	100	D	5	6	8	7	7
		D	9	12	14	14	18
		D	10	9	9	18	10
		D	7	6	6	5	7
Menadione sodium bisulfite, oral	60	D	10	10	12	10	20
		D	6	5	-	5	5

* CPID = 2-p-chlor-phenyl-1,3-indanedione. D = dicoumarol.

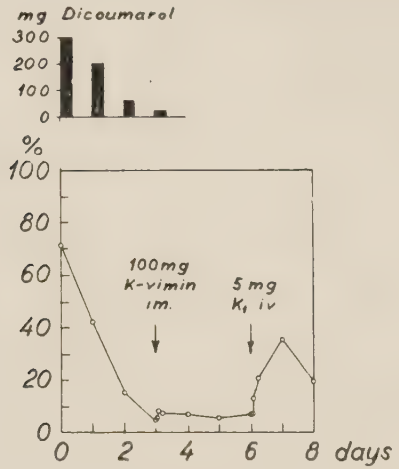


Fig. 1. Man, 79 years. Intramuscular (im.) and intravenous (iv.) administration of 2 different forms of vitamin K.

Abscissa: days after first dose of dicoumarol.
Ordinate: prothrombin-proconvertin concentration (PP-value) in per cent of normal.

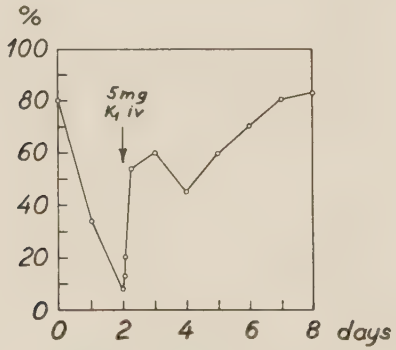


Fig. 2. Man, 70 years. Intravenous injection (iv.) of vitamin K1

Abscissa: days after single dose of 20 mg 2-p-chlorphenyl-1,3-indanedione.

Ordinate: prothrombin-proconvertin concentration (PP-value) in per cent of normal.

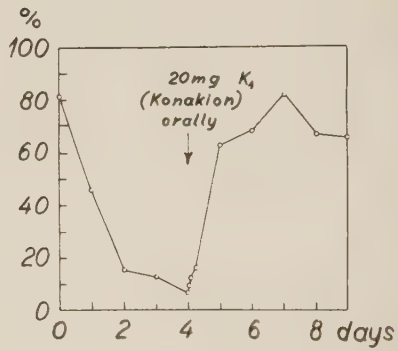


Fig. 3. Man, 84 years. Oral administration of vit min K1 (Konakion, Roche).

Abscissa: days after single dose of 20 mg 2-chlorphenyl-1,3-indanedione.

Ordinate: prothrombin-proconvertin concentrati (PP-value) in per cent of normal.

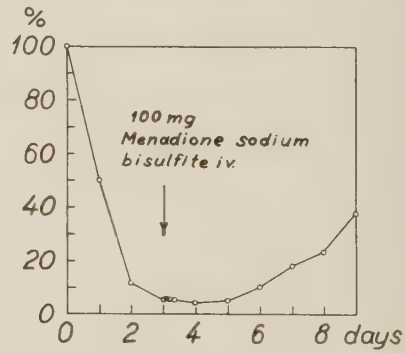


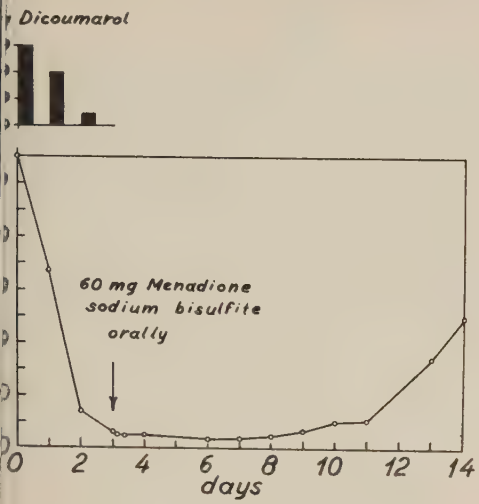
Fig. 4. Woman, 84 years. Intravenous injecti (iv.) of menadione sodium bisulfite.

Abscissa: days after single dose of 20 mg 2-chlorphenyl-1,3-indanedione.

Ordinate: prothrombin-proconvertin concentrati (PP-value) in per cent of normal.

coagulant treatment was discontinued when vitamin K was given.

We therefore recommend intravenous administration of 10 to 20 mg of a sterile aqueous colloidal solution of vitamin K when treatment with dicoumarol-like ant coagulants has resulted in serious hemo rhage. The high doses used by other i



5. Man, 86 years. Oral administration of menadione sodium bisulfite.

scissa: days after first dose of dicoumarol.
nate: prothrombin-proconvertin concentration (PP-value) in per cent of normal.

igators are unnecessary and can be gerous by favoring thrombosis. In hospi-ed cases without hemorrhage, where the -value has fallen below 10 per cent, it is ally sufficient to discontinue the anti-gulant treatment temporarily. In similar oulatory cases vitamin K_1 treatment as cribed is advised because the patients are under constant medical supervision. ording to our earlier and present experi-a lowering of short duration of the PP-e below 10 per cent in the beginning of treatment with anticoagulants does not rd any great risk of spontaneous hemor-ge.

reatment with menadione and its simpler vatives must be considered of no value use of the low and uncertain efficiency these compounds in counteracting the oprothrombinemia caused by dicoumarol anticoagulants. We do not agree with the views of Hilden & Munck (1953) and Wright, Beck & Marple (1954) who recommend menadione as an antidote against dicoumarol anticoagulants.

SUMMARY

1. Doses of about 10—20 mg of vitamin K_1 given intravenously in the form of a sterile aqueous colloidal solution are recommended as a means of raising the proconvertin-prothrombin value in cases where treatment with dicoumarol anticoagulants has resulted in serious hemorrhage.

2. In this way a therapeutic effect is obtained within 2 hours, whereas oral administration acts more slowly. Higher doses are unnecessary and dangerous by favoring thrombosis.

3. Treatment with menadione and its simpler derivatives is considered of no value because of the low and uncertain efficiency of these compounds in counteracting the hypoprothrombinemia caused by dicoumarol anticoagulants.

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VITAMIN A AND CAROTENOID LEVELS IN BLOOD SERUM WITH SPECIAL REFERENCE TO VALUES OBSERVED IN DISEASES OF THE LIVER

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During the past few decades the vitamin A and carotene contents of serum in health and disease have been studied by various authors. The values observed have varied, probably as a result of varying technique used by the different investigators.

The principal carotenoids in serum are carotene and xanthophyll, which are present in a relatively fixed proportion. Measurement of total carotenoids may therefore serve as a practical index of carotene content.

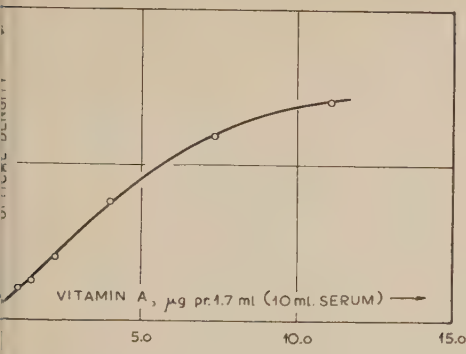
The normal vitamin A range in serum is usually reported as 100 to 300 international units per 100 ml (1 I. U. = 0.34 μg vitamin A acetate[1]), and the normal carotene range as 100 to 300 μg per 100 ml.

It is an established fact that the concentrations of vitamin A and carotene in serum under pathological conditions may show considerable deviation from normal, irrespective of the vitamin supply (Lindquist, 1938; With, 1940; Haig & Patek, 1942; White, Bone, Ruffin & Taylor, 1950). Acute infectious diseases are known to be associated with a decrease of the vitamin A content during the initial stage, to be followed by a rapid rise during the convalescent period (Lindquist, 1938). Increased vitamin A levels have been demonstrated in patients with chronic nephritis (Lindquist 1938). Following administration of large doses of vitamin A Kagan, Thomas, Jordan & Abt (1950) found a considerably higher rise of the serum values in children with nephrosis than in normals. It is spectacular that consumption of alcohol causes a transient increase of the vitamin A level (Clausen, Breese, Baum McCoord & Rydeen, 1940). Pett (1940) had previously demonstrated a reduction of the adaption time following ingestion of alcohol.

In most cases of acute hepatitis Lindquist (1938) found decreased vitamin A levels during the initial stage of the disease, followed by a rapid increase of the values during the convalescent period. Similar observations were made by White & coworkers (1950). Haig & Patek (1942) in a study of patients with Laennec's cirrhosis found abnormally low levels in 92 per cent among those with "decompensated cirrhosis". In patients with obstructive jaundice, on the other hand, White & coworkers (1950) in most instances found vitamin A levels ranging within normal limits.

The present study has been carried out for the purpose of elucidating the possible value of vitamin A and carotenoid estimations as a diagnostic aid in diseases of the liver.

[1] World Health Organization: Tecnical Report Series No. 3, 1950.



1. Calibration curve for vitamin A estimation. lyn's photo-electric macrocolorimeter. Filter 20. Values calculated as vitamin A acetate.

METHODS

he vitamin A content of serum was de- ined by the Carr-Price reaction, mainly odified for serum analyses by Dann & lyn (1938). As measuring apparatus was l an Evelyn photo-electric colorimeter elyn 1936) and a filter with transmittance imum 620 mμ. The saponification was mplished according to Vavich & Kem- er (1950) and the extraction according Dann & Evelyn (1938). The residual r ether extraction was dissolved in an- rous chloroform, whereupon the Carr- e reaction was performed.

he concentration of carotenoids was sured directly in the chloroform extract, g wavelength 420 mμ. Then results could read directly from a calibration curve d on several measurements of known centrations of β-carotene.

he calibration curve for the Carr-Price tion was obtained by use of solutions of S. P. Vitamin A Reference Standard in anhydrous chloroform (Fig. 1).

he values obtained with the reaction in m extracts must be corrected for the interference of carotenoids which react with antimony trichloride to produce a blue pigment. This is done by means of the formula:

$$C_a = A - \frac{C\,k}{20}$$

C_a is the actual concentration of vitamin A and A that of apparent vitamin A by the Carr-Price reaction, both expressed as μg of vitamin A acetate per 100 ml of serum. C_k = Concentration of carotenoids expressed as μg per 100 ml of serum.

Blood to be tested was usually drawn before breakfast. In most instances the analysis was carried out within eight hours. In some instances the separated serum was stored overnight in darkness at 4° C. Such storing did not influence the results significantly.

RESULTS

Carotenoid and Vitamin A concentrations in serum were measured in 100 normal individuals, 50 men and women. The average carotenoid level was 97 μg per 100 ml serum, the average vitamin A level was 27.7 μg per 100 ml. The results are shown in Fig.s 2 and 3.

Highest mean vitamin A levels were observed in men (Fig. 3). Mean values in 50 men 29.6 μg per 100 ml. Mean value in 50 women 25.9 μg per 100 ml. Highest mean carotenoid levels were observed in women. Fig. 2. Mean value in 50 men 91.5 μg per 100 ml. Mean value in 50 women 102.5 μg per 100 ml.

Of the 100 normal individuals investigated, 6 had received addition of vitamin A to the diet. The average level of carotinoids and vitamin A in these individuals did not

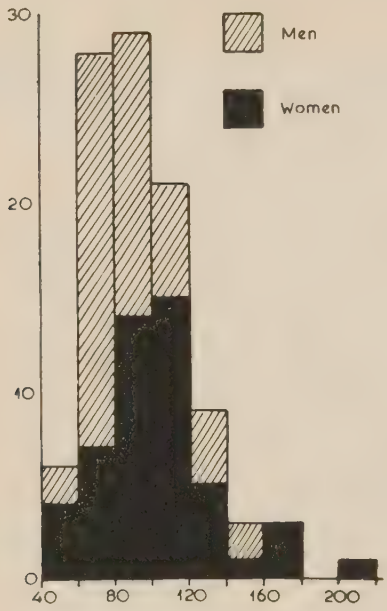


Fig. 2. Concentration of carotenoids in serum in 100 adult normal individuals. Abscissa: μg of carotenoids per 100 ml. Ordinate: Number of individuals.

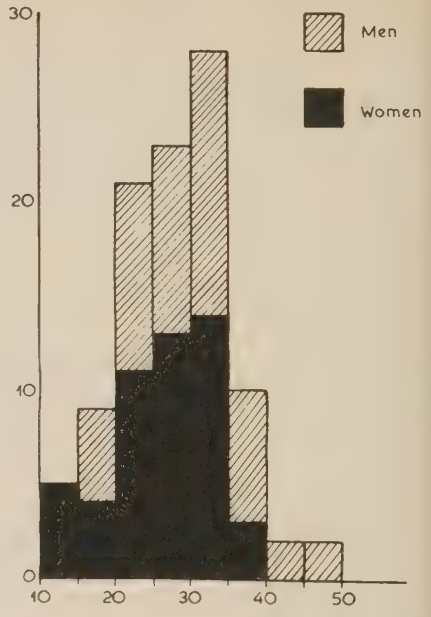


Fig. 3. Concentration of vitamin A in serum i 100 adult normal individuals. Abscissa: μg c vitamin A per 100 ml. Ordinate: Number of individuals.

deviate from the overall average. All these individuals seemed to have been living on a balanced, sufficient diet. None of them had consumed alcohol within the day before the sample was drawn.

Vitamin A and carotenoids were also determined in 128 patients suffering from the following diseases:

Chronic alcoholics	18
Various febrile diseases	12
Steathorrhea	5
Uremia	13
Renal diseases without uremia	6
Hepatic and biliary diseases	74

The 18 chronic alcoholics had been addicted for years. The vitamin A levels observed in this group averaged 30.8 μg pe 100 ml, the carotinoid values averaged 57. μg per 100 μg serum (Fig. 4). There wa a considerable reduction of the carotenoi level, while the concentration of vitamin A was normal, with a certain trend toward increased values.

In 12 individuals suffering from vario febrile diseases, the vitamin A level as de termined during the acute phase average 19.3 μg and the carotenoids 59.4 μg. Bot the values were definitely decreased.

Of 13 patients with uremia resulting fro bilateral renal cysts, chronic nephriti chronic pyelonephritis and diabetic nephr pathia, the major proportion showed de finitely increased vitamin A values. Th

4. Concentrations of carotenoids and vitamin n serum in 18 alcoholics. Abscissa: μg per 100 of serum. Ordinate: Number of cases. Dotted curve: Normal levels.

otenoid values were normal (Table I). a single patient with arthritis urica and reased blood urea concentration (70 mg 100 ml) the concentration of vitamin A 37.4 μg per 100 ml serum, the carotenoid ue 52 μg per 100 ml.

n 6 patients with renal disease without mia the carotenoids and vitamin A were h within normal limits, 98.3 $\pm$ 23, 27.5 3.7.

b l e I. *Concentrations of vitamin A and rotenoids in serum in uremia resulting from chronic renal diseases.*

Diagnosis	Number of estimations	Vitamin A μg/100 ml	Carotenoids μg/100 ml	Blood urea mg/100 ml
Bilat. ren cyst.	1.	56.8	85	113
»	1.	31.7	41	185
Chron.nephritis.	1.	42.7	147	78
»	2.	42.3	61	207
»	3.	66.8	104	104
»	3.	60.4	82	91
»	1.	101.5	70	94
»	4.	163.7	77	154
»	1.	44.8	158	46
»	2.	50.0	128	114
»	2.	40.4	51	77
Pyelonephritis.	1.	102.8	44	202
Diab.nephropatia.	1	52.4	140	68

Levels measured in patients with diseases of the liver or biliary passages are shown in Figs. 5 and 6.

In the group of hepatic and biliary diseases, vitamin A and carotenoids were measured in a total of 74 patients. In 14 of them, suffering from cirrhosis, definitely decreased vitamin A levels were observed, the levels in all of them being below 20 μg per 100 ml of serum. The lowest value observed was 3.6 μg per 100 ml (Fig. 5). The carotenoids were on the whole only moderately decreased but showed considerable individual variations. Three patients with chronic hepatitis all showed decreased Vitamin A levels, but carotenoid level in all instances within normal limits.

In 8 patients with obstructive jaundice the vitamin A level varied within normal limits, averaging 26.1 μg per 100 ml. In this group the carotenoids were considerably below normal.

Acute hepatitis was generally associated with decreased values for both vitamin A and carotenoids. In these cases however the estimations were made without regard to the stage of the disease, for which reason the results are not conclusive.

Seven patients with cholecystitis and seven with uncomplicated gall stone all had normal vitamin A levels, while a decrease of the carotenoids was observed in those with cholecystitis.

In 16 patients with secondary carcinoma of the liver, the concentration of vitamin A varied within wide limits, viz., from 5.1 μg to 42.3 μg per 100 ml. The average level, 17 μg per 100 ml, however was below normal. A definite decrease of the carotenoids was also noted in this group.

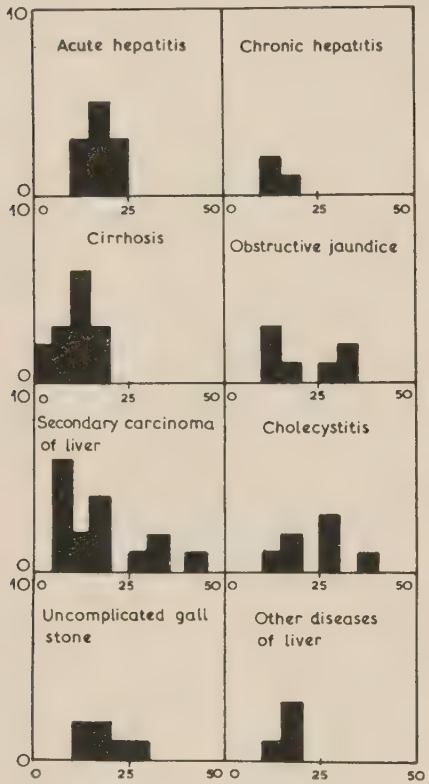


Fig. 5. Concentrations of vitamin A in patients with diseases of liver and biliary passages. Abscissa: μg per 100 ml of serum. Ordinate: Number of cases.

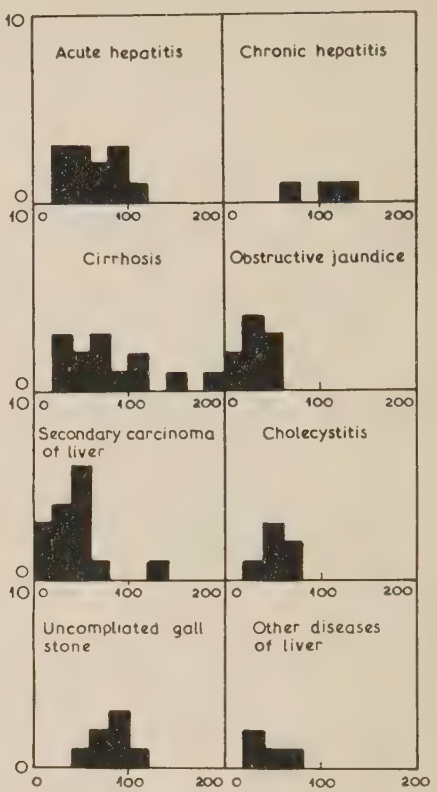


Fig. 6. Carotenoids in patients with diseases of liver and biliary passages. Abscissa: μg per 100 ml of serum. Ordinate: Number of cases.

The group of "other diseases of the liver" includes four cases. One was a case of fistula of the common bile duct, another suffered from hemolytic jaundice, while the remaining two suffered from jaundice of unknown origin.

DISCUSSION

In this study vitamin A and carotenoid estimations have been performed in serum from normal individuals and a number of patients for the purpose of obtaining values for comparison with those obtained in patients with diseases of the liver.

Normal averages in this series appear to be lower than those usually stated in the literature. This applies to both vitamin A and carotenoids, particularly the latter. Week & Sevigne (1950) in a group of 18 normal men estimated the average vitamin A content to 53.2 μg per 100 ml. In a series of 7 women they estimated it to 41.2 μg per 100 ml. The average level observed in the present series was 27.7 μg per 100 ml and our studies seem

indicate that the normal vitamin A range serum is 15 to 45 μg. Like Week and vigne, we found somewhat higher values men than in women. Whether or not this ference is real, is not evident from this ies. With found an average content of otenoids in serum of about 100 μg per 0 ml. From our series it seems justified conclude that the normal carotenoid range from 50 to 100 μg.

Abnormally high concentrations of vitamin A in serum were demonstrated in cases suffering from chronic renal diseases with uremia. Moderately increased values were observed in chronic alcoholics. The carotenoid values in both instances were slightly below normal.

A survey of the values observed in patients with diseases of the liver and biliary passages is given in Table II.

Table II. *General survey of vitamin A and carotenoid levels in patients with diseases of the liver and biliary passages.*

Disease	Vitamin A	Carotenoids
Cirrhosis	Highly decreased	Moderately decreased
Acute hepatitis	Decreased	Decreased
Chronic hepatitis	Decreased	Normal
Secondary carcinoma	Moderately decreased	Highly decreased
Obstructive jaundice	Normal	Highly decreased
Cholecystitis	Normal	Highly decreased
Uncomplicated gallstone	Normal	Normal

t is generally believed that the absorption provitamin A as well as the vitamin itself avored by simultaneous resorption of fat. is presumption was not confirmed as far vitamin A is concerned. Patients with atorrhea in this series showed no signiant decrease of the serum values. The otenoids, on the other hand, were often siderably decreased in the same patients. tients with obstructive jaundice all showed lefinite decrease of the carotenoid level, ile the concentration of vitamin A was significantly decreased. These results m to indicate that the carotenoid content serum is closely related to absorption, ile the vitamin A in serum is but slightly luenced by the absorption of fat or by the retion of bile to the intestine.

Like the above mentioned authors we found highly decreased levels for vitamin A in the presence of cirrhosis and chronic hepatitis far lower than in any other diseases studied. The carotenoid level under similar circumstances, however, came close to normal. In the presence of secondary carcinoma of the liver, vitamin A ranged within wide limits but with a trend towards subnormal values. This condition however, in contrast to cirrhosis and chronic hepatitis, was associated with a low content of carotenoids.

As to acute hepatitis, the results cannot be considered representative in view of the fact that consideration was not given to the actual stage of the disease.

The fact that damage of the liver parenchyma is associated with a low vitamin A

content in the presence of a normal concentration of carotenoids seem to indicate that the actual synthesis of vitamin A takes place in the liver, and that this mechanism will suffer in the presence of parenchymal involvement. A reduced absorption of carotenoids as observed in obstructive jaundice, on the other hand, does not involve a fall of the vitamin A content, indicating that carotenoids are usually present in excessive amounts.

SUMMARY

Estimations of concentrations of vitamin A and carotenoids in serum have been performed in 228 individuals.

Values obtained in 100 normal individuals indicate that the normal vitamin A level in serum is 15—45 μg per 100 ml, and the normal carotenoid level 50—200 μg per 100 ml.

A considerable increase of the vitamin A content was observed in patients suffering from chronic renal disease with associated uremia. Slightly increased values were observed in alcoholics.

Studies were made with a view to elucidate the value of vitamin A and carotenoid estimations for the diagnosis of diseases of the liver. Occlusive jaundice was found to be associated with a definite decrease of the carotenoid level without significant decrease of the vitamin A content. Chronic parenchymal lesions of the liver, on the other hand, were associated with a definite decrease of the vitamin A content while the content of carotenoids varied within normal limits.

In patients with secondary carcinoma of the liver highly varying values were observed although a trend toward subnormal values for both vitamin A and carotinoids seemed evident.

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PERCUTANEOUS CATHETERIZATION OF PERIPHERAL ARTERIES AS A METHOD FOR BLOOD SAMPLING

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Arterial pressure measurements and blood mpling are common procedures in the prac- of clinical physiology. In cardio-pul- nary function tests and in cardiac cathe- ization the problem of obtaining repeated erial blood samples is of prime impor- ce. The methods most commonly used the puncturing of a peripheral artery eral times with a thin needle or once with indwelling needle ad modum Cournand. is technique is only a partial solution of problem since the patient is immobilized order to avoid pain and injury of the ery and it is often impossible to obtain a nquil state.

Fitzpatrick, Schnabel & Peterson (1949) sented a procedure for vascular catheteri- ion by introducing thin polyvinal catheters ough the needle after percutaneous punc- e. Seldinger (1953) described a technique percutaneous catheterization of vessels h catheters of the same dimensions as the ncture needle used. The advantage of the ter method, besides the considerable de- ase in the diameter of the needle required, that no indwelling needle is necessary to vent bleeding at the side of the catheter be- se it fills up the puncture hole completely. us it is possible to leave the catheter in a long time without extra discomfort to the patient, who is able to move the arm freely. Seldinger's method has formerly been used only in roentgenological practice. This paper is a report of the experiences of a modification in physiological and clinical practice.

MATERIAL

The method has been used in about 140 cases in the Clinical Physiological Laboratory in connection with cardiac catheterization, pulmonary function tests and determinations of the cardiac output with Evans blue dye. In the Roentgen Diagnostic Department about 350 catherizations have been performed for aortography and angiography of peripheral vessels.

EQUIPMENT

The original device described by Seldinger (1953) has been used with some modifications. The most suitable size of the needle for blood sampling purposes is No. 160 (external diameter 1.5 mm). The puncturing set consists of an inner sharp needle with an obturator and an outer blunt cannula. The needle should be about 1 mm longer than the cannula. The catheter adapter for attachment of a syringe has been furnished with a stopcock to make it possible to fill catheter with

a solution of heparine-saline to prevent coagulation instead of using a continuous drip. The flexible part of the original leader was flaccid only three cm from the tip. Because of complications (se below), we have used a leader that is flaccid except in the distal 4—5 cm. It is important that the catheter is closely adapted to the leader to avoid extra damage to the artery. Figure 1 shows a simple procedure for ensuring this.

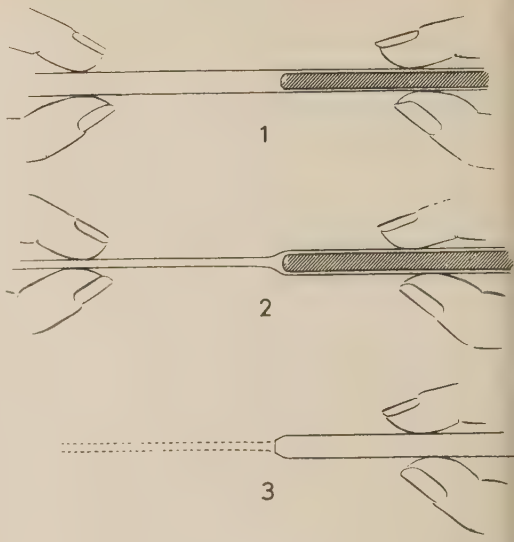


Fig. 1. The procedure of molding the tip of the catheter to achive smoother introduction into the artery.

1. Insert leader into the catheter leaving its tip a few cm from the tip.
2. Pull the unfilled portion thus adapting the catheter to the leader.
3. Cut off the catheter close to the tip of the leader.

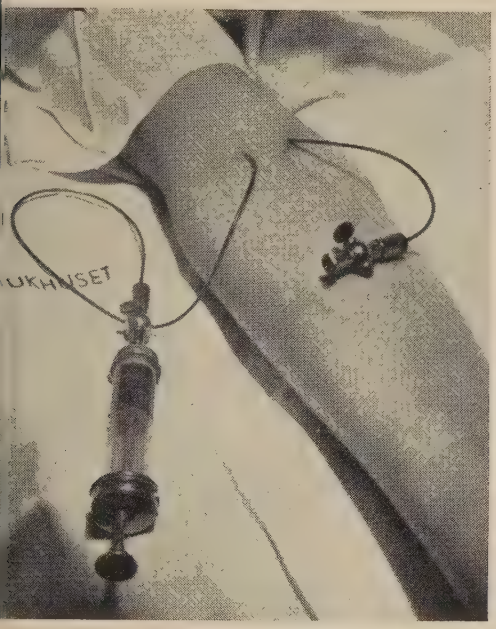
CHOICE OF ARTERY

It is well established fact that *the brachial* artery is most suitable for puncturing. It is easy to localize and there is as a rule little subcutaneous fat in that region. The artery can be fixed satisfactorily between the fingers, and the needle is without difficulty pushed further into the artery after the puncturing. The most important advantage is the good opportunity to compress the artery after puncturing, first manually and then with a bandage over sponge.

PROCEDURE FOR PUNCTURING

The skin of the whole arm is washed with 0.5 per cent iodine in diluted ethanol. The artery is palpated with the index and middle fingers just proximal to the antecubital fossa, where it passes under lacertus fibrosus, and the direction of the artery is established. The arm is slightly hyperextended by inserting a pad under the elbow and the hand pronated. The skin and the periarterial tissues are anesthesized with 2—3 cc 0.5 per cent xylocain (diaethylamino-acet-2.6-xylidid.HCl. Astra Sweden). The puncture and catheterization are performed in the same manner as described by Seldinger. It is very important to push the needle far up in the artery after puncturing, before the leader is introduced, to ensure that the direction of the needle is the same as that of the artery.

When the catheter has been pushed far enough into the artery the leader is withdrawn and the catheter is filled with a heparine-saline solution. The catheter can be left in the same artery for a considerable time. In some cases of poliomyelitis the indwelling arterial catheter has been left for fourteen days without complications. In these cases it is necessary to flush the catheter



g. 2. The ulnar catheter is within the brachial ery. The radial catheter is within a vein, with the tip in the right auricle.

th the heparine solution at least four times day. If the catheter is not patent when ing this, it is important not to inject the lution but to try to suck out the coagulum, order to avoid an embolus. Otherwise the theter is withdrawn. After each blood mple the catheter should be flushed with parine solution.

When the investigation is finished the heter is removed and pressure is applied er the artery, first manually for 5—15 nutes, then by means of a bandage over sponge. This bandage should be left for -4 hours and is then replaced by a small ce of tape. The older the patient, the ger should the artery be compressed. The ient should be told not to carry anything with that arm during the first day. Figure 2 demonstrates two percutaneous catheters, the ulnar catheter is within the brachial artery, the radial one within a vein.

COMPLICATIONS

The most common complications are hematomas and spasm in the artery.

The amount of *hemorrhage* is dependent on the technique, the amount of arteriosclerosis and the arterial blood pressure. If the artery is punctured and the catheter introduced at the first attempt the leakage after withdrawal of the catheter is minimal. Elderly patients with sclerotic vessels, with or without hypertension, are apt to get larger hematomas than others. A hematoma is as a rule a complication of little importance. In about 180 cases, after catheterization of the brachial artery, there was no serious hematoma in any case. In more than 150 gynaecological cases, in which abdominal aortography after catheterization from the femoral artery with this method was performed, the tendency to formation of a hematoma could be studied at operation. The hematoma was in no case of importance and was never found anywhere else than in the subcutis. These patients were told, after careful compression of the artery for about ten minutes, to lie in bed for at least 2 hours.

It appears from this, that if the technique is good, the risk of a hematoma of any importance is minimal. If, after an unsuccesful attempt at puncture, an arterial hematoma appears, further attempts would be difficult, and the risk of hematoma formation increased considerably by further damage to the artery. It is significant that the only hematoma of clinical consequence appeared

in one of the cases where the attempt at catheterization did not succed (see case report). The hematoma was large enough to cause shock and peritonitis. When larger hematomas appear, those arising from the femoral artery are more difficult to control than those arising from the brachial artery. Therefore, puncture of the femoral artery should be avoided in ambulant patients, especially elderly ones. Patients with hematomas should be treated with antibiotics to prevent infections.

Spasm occurs in almost all patients to some degree and seems to be most common in females. As a rule the spasm disappears after a while. Only in cases of severe injury of the artery will the spasm remain long after the catheter has been withdrawn. This spasm should be treated with repeated injections of papaverine, warmth locally, and if necessary with blocking of the stellate ganglion.

CASE REPORTS

Case 1. A man 19 years of age with poliomyelitis, totally paretic and lying in a respirator. Eighteen hours after an unsuccessful attempt to catheterize the right femoral artery the patient showed signs of peripheral shock. The systolic blood pressure fell below 50 mm Hg. The cause of the shock was an enormous hematoma apparent in the right groin, at the site of catheterization. Prothrombin index was 0. The patient was immediately treated with transfusions of blood and Macrodex in combination with a noradrenaline drip. K-vitamin and protamine-sulfate was administered. The day after, the patient showed signs of peritonitis and subileus. The hematoma evidently had dissected along the psoas to the retroperitoneal space. A resistance was found to the right on palpation of the abdomen. A roentgenogram showed an opacity at the site of the right psoas contour. The patient improved after some days. The cause of the low prothrombin index is still obscure.

Case 2. A girl, 16 years old, suffering from poliomyelitis with respiratory palsy. She was catheterized in the right brachial artery. The catheter remained in the artery for 3 days. By mistake it was not flushed with heparine-saline solution for 36 hours. The patient complained of local pain in the antecubital fossa. There was a local hyperemia around the site of the puncture and a periarterial infiltration was palpable. An attempt to suck blood through the catheter was unsuccessful. The catheter was removed. The pulse in the radial artery was not palpable. The right hand was colder than the left but of normal color. The patient had no paresthesia in the arm and no other signs of arterial occlusion. The complication was regarded as a result of local spasm caused by reaction in the periarterial tissues and was treated with warmth locally, papaverine and heparine. Four days afterwards she was vithout any symptoms of spasm.

Case 3. A woman, 42 years of age, with stenosis of the pulmonary artery. During catheterization the flaccid tip of the original leader became wedged in the artery and could only be removed by using force. Afterwards the patient was pulseless in the radial artery and cold peripherally in the arm with slight paresthesia of the hand. This complications was considered to be the result of local spasm. The patient was treated with repeated injections of papaverine and warmth locally. There were no signs of spasm on the following day.

DISCUSSION.

A study which requires the patient in a tranquil state, such as cardiac output and pressure measurment, makes it impossible to perform an arterial puncture close to the investigation. By using and indwelling needle a. m. Cournand this problem is only partly solved. The feeling of beeing immobilized in fact causes the patient uneasiness. Hyperventilation caused by pain from an arterial puncture is known to alter the blood gas content.

The method described for arterial cathe-
zation eliminates these difficulties. When
catheter has been introduced, the samples
be obtained without causing the patient
pain and his arm can be moved without
harm. The small diameter of the catheter
bles blood to pass freely to the distal parts
he artery. As a rule the pulse at the wrist
alpable, when the catheter remains in the
chial artery.

Experience with the method in about 500
es shows that catheterization is easy to
form and, in the main, free from com-
ation in this material. The method has
been practised for percutaneous cathe-
zation of the caval veins.

The procedure should have a wide applica-
n in clinical practice. Patients who need
avascular drips and those who are sub-
ted to repeated blood sampling (venous
arterial) will suffer less if the procedure
cribed is practised. The device might be
d preoperatively in cases carrying a great
k of shock. Percutaneous catheterization
f value when performing exchange trans-
ions. Thoracic aortography can in this
y be performed without any incision and
hout ligature of the peripheral artery.
other application of the mehod has been
ctised in poliomyelitis. For the control
respiration, repeated arterial blood ana-
es are often necessary and for this reason
blood sampling should be simple to per-
form. The method described with an indwelling polyethylene catheter has turned out to be of great value also in this respect. The catheter has functioned for two weeks in some cases of poliomyelitis.

The experience gained seems to justify the introduction of the method in the clinic as a routine.

SUMMARY

1. The importance of taking arterial blood samples with the patient in a tranquil state when determining the cardiac output is stressed.

2. A method using a polyethylene catheter for this purpose is described.

3. The equipment, choice of artery and technique of puncture are discussed.

4. Some complications are discribed.

5. The method is suitable for laboratory and clinical practice.

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SOME REMARKS ON GENTISINAMIDE AND ITS THERAPEUTIC VALUE

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INTRODUCTION

Sodium salicylate has been therapeutically used since 1875 and acetylsalicylic acid since 1899. Both can cause irritation of the gastric mucosa, tinnitus and respiratory disturbances. Salicylamide, which was introduced some years ago, has about the same analgetic and antirheumatic value but shows hardly any disturbing side-effects and is for that reason of much value, when other salicylates are not tolerated (Harthon & Sigroth 1952, 1953).

Kapp and Coburn (1952) found gentisic acid as a metabolite of salicylic acid, and this substance was observed to exert a more powerful inhibiting action on hyaluronidase than salicylic acid. Since Meyer and Ragan (1948) suggested that an increased hyaluronidase activity was a possible mechanism in the pathogenesis of rheumatic diseases, sodium gentisate was tried against rheumatic fever and rheumatic arthritis. The clinical improvement was rapid and no toxic reactions were noted. These, results were confirmed by Camelin, Accoyer, Pellerat, Lafuma & Coirault (1949), Camelin, Steiger, Morel & Tary (1950) and Schaefer, Rashkoff & Meigbow (1950). On the contrary Rosenberg, Krevsky & Kagan (1952) and Batterman & Sommer (1953) found only a transient improvement. Clarke, Mosher & Clarke (1953) and Clarke & Mosher (1953) have tested sodium gentisate, alone and combined with anion exchange resin or methylcellulose, and the ethanolamide of gentisic acid. They are of the opinion that compounds of gentisic acid are more effective in surpressing the manifestations of rheumatic fever than other therapeutic remedies.

As we had tested the clinical value of salicylamide we were also interested in studying the therapeutical effects of the amide of gentisic acid. As far as we know, there is no earlier report of its metabolism or clinical value, though Becher, Miksch, Rambacher & Schäfer (1952) used gentisinamide in experiments on themselves. They proved it to be a metabolite of salicylamide, an observation earlier made on rabbits by Bray, Ryman & Thorpe (1948).

CHEMISTRY

Gentisinamide is 2.5-dihydroxybenzamid and can be prepared analogically to other acid amides from the corresponding ester with aqueous ammonia.[1] It is a slightly

[1] We are indebted to Dr. B. af Ekenstam for the generous supply of the substance used in this investigation.

ow, odorless and tasteless crystalline sub-
ce, sparely soluble in water, m.p. 218° C.
s not stable in alkaline solutions, being
dly oxidized to a brown cinoid com-
nd. The infrared and ultraviolet spectra
recorded in Figs. 1 and 2. In ultraviolet
t gentisinamide shows a strong green
rescence as distinguished from the blue
of gentisic acid.

he quantitative determination of genti-
mide is possible by the aid of its ultra-
et absorption, by a bluish complex com-
nd formed with Fe^{3+}, a reaction common
ost phenolic compounds, and by a reac-
with the Folin-Ciocalteau reagent which
ned to be most suitable in our experi-
ts. This reagent can be used either in
line or in acid solutions. The acid reac-
, however, eliminates the risk of un-
ted effects due to air oxidation. It also
inguishes gentisates from compounds of
ylic acid, which do not react in acid
tion. In all experiments we have hydro-
d the sample prior to determination
gh the reagent gives the same reaction
unhydrolyzed gentisinamide as with
isic acid.

TOXICITY

he acute and chronic toxicity to rats has
shown to be low (T. Teorell: Private
munication). During the preparation of
paper LD_{50} on rats is reported to be
mg/kg (Way, Takemori, Smith, Ander-
& Brodie 1953) when injected intra-
toneally. As nothing has been published
ut the metabolism of gentisinamide, we
e determined the serum level and urinary
retion in man after administration of a
le dose.

CHEMICAL METHODS

To 1.0 ml of serum or urine in a 2 ml vial 0.5 ml of 7-N sulfuric acid was added and the vial fused. The content was then hydrolyzed by heating for two hours at 115° C. The vial was allowed to cool and then crushed with a glassrod in a separatory funnel. The mixture was extracted with 20 ml of diethylether and 10 ml or a suitable aliquote of the ether phase reextracted with 6 ml of 5 per cent sodium hydrogen carbonate. To 5 ml of the aqueous layer 1 ml of concentrated hydrochloric acid was added, drop by drop. When the carbon dioxide had expelled, 1 ml of the Folin-Ciocalteau reagent was added and the whole made up to 10 ml with distilled water. The green color obtained was read in a Beckmann DU Spectrophotometer at 750 mμ against a blank of the phenolic reagent prepared in the same manner.

A standard curve was obtained by dissolving known amounts of gentisinamide in serum, and treating the samples in the same manner.

PAPER ELECTROPHORESIS

0.03 ml of serum from a person who had taken gentisinamide was placed on several strips of Whatman filter paper No. 1 soaked in Veronal buffer of pH 8.6. The strips were placed in a paper-electrophoresis apparatus and run for 6 hours at 300 volts. After drying, one of the strips was colored with bromphenol blue. A similar strip not colored was studied for fluorescence under an ultraviolet lamp and the location of fluorescence compared with the location of the different protein fractions.

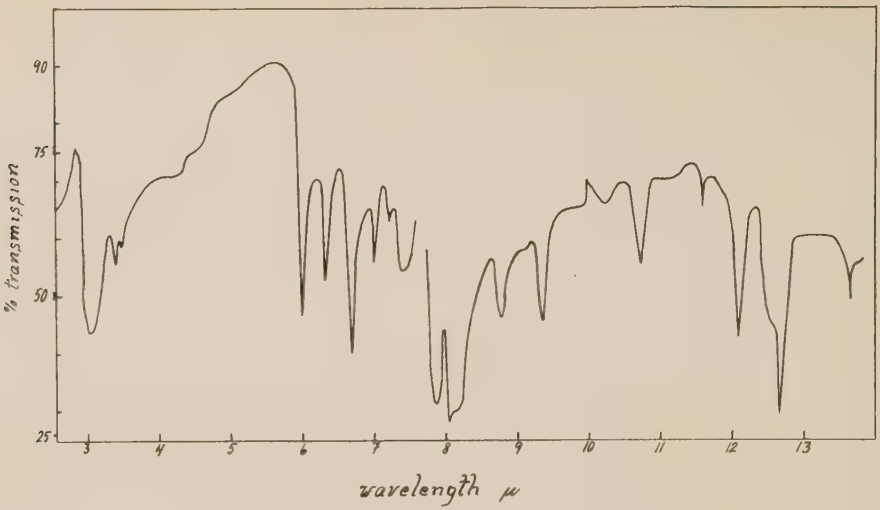

Fig. 1. Infrared spectrum of gentisinamide. Florolube S (polyfluorethylene) mull to 7.70 μ, longer wavelengths paraffin mull. (Perkin-Elmer Model 12 C spectrophotometer.)

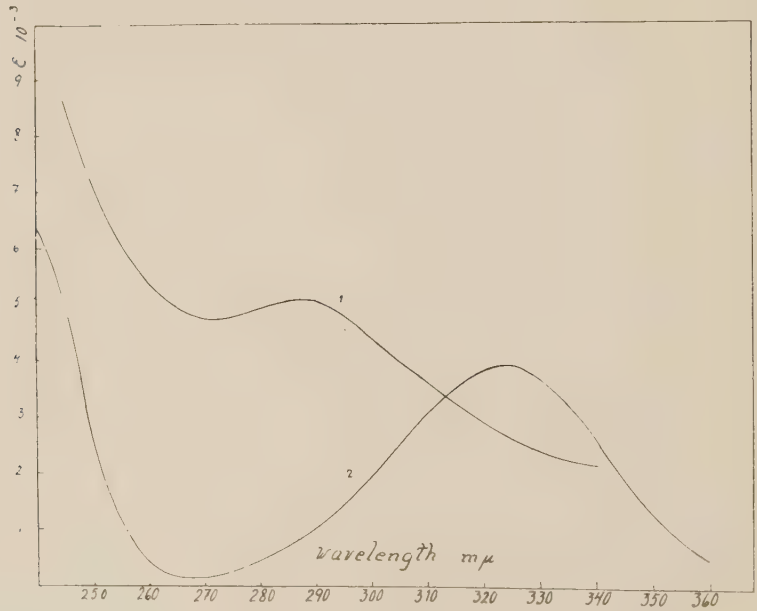

Fig. 2. Ultraviolet spectra of gentisinamide: 1) in 0.05 N sulphuric acid, 2) in borate buffer containing 10^{-3} M. sodium sulphite, pH 11.2. Concentration of gentisinamide 10^{-4} M.

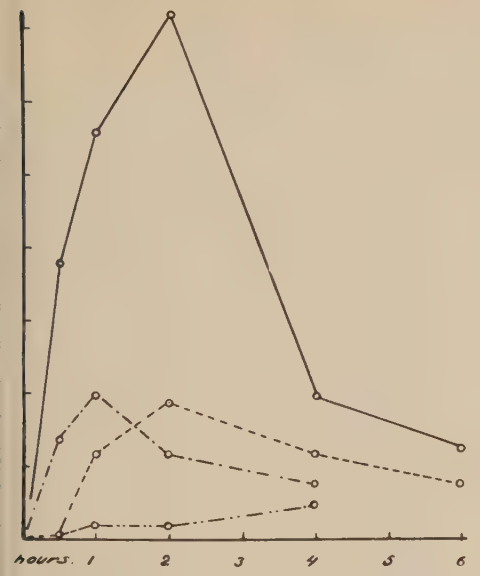

Fig. 3. Plasma levels of gentisinamide.
—— After ingestion of 4.5 g of gentisinamide on resting stomach.
— — The same dose after a light meal.
— — After ingestion of 1.5 g of gentisinamide on resting stomach.
. — The same dose after a light meal.

EXPERIMENTAL RESULTS

entisinamide was given by mouth in a le dose of 1.5 and 4.5 g respectively to healthy females. The experiment was ormed in the postabsorptive state and repeated several days later after break- (porridge and milk, bread, butter and ee). Venous blood was then drawn from rm vein at intervals and serum analyzed gentisate content. The results are pre- ed in Fig. 3.

his figure shows that it is impossible to in a high serum concentration if the ent has eaten before taking the drug. lose of 1.5 g of gentisinamide gives a apeutically negligible serum level. If the same dose is taken in the postabsorptive state, the serum concentration is at least four times higher. The same difference is observed after the higher dose.

The urinary output of gentisic compounds was determined in two experiments and found to be 44 per cent in 8 hours of the dose taken by mouth and 61 per cent in 24 hours. These figures are in accordance with those found by Clarke, Mosher & Clarke (1953) for gentisic acid and sodium gentisate.

Ungar, Damgaard & Wong (1952) have investigated the ultraviolet absorption spectrum of gentisic acid and observed a shift and increase of the absorption maximum in the presence of serum. It had earlier been assumed that the acid is "converted" as soon as it comes in contact with serum (Meyer & Ragan, 1948).

Electrophoresis of serum from persons who had taken 4.5 or 7.5 g of gentisinamide three hours before blood was drawn showed a strong fluorescence located to the albumin spot. No fluorescence could be detected at any other place on the strip. When gentisinamide in water solution was studied electrophoretically in the same manner, it was found to move slowly in the same direction as the proteins. However, the fluorescence was reduced gradually due to oxidation in the alkaline buffer, and it had disappeared completely in about two hours.

From these experiments one may conclude that gentisinamide in blood is at least partly combined with the albumins and that this combination seems to prevent the gentisinamide from oxidation at pH 8.6. Thus gentisinamide does not seem to combine with the globulin fractions. It can, however, do so in such a manner that the fluorescence is

quenched or the substance oxidized. The experiments give no information whether the gentisinamide exists in free form in serum. To make the behavior clearer, a closer study is necessary.

CLINICAL RESULTS

Twenty patients with various algetic diseases have been treated with gentisinamide, from four days to one month. The dosage has varied from 3.0 to 7.5 g a day divided in three to five doses at regular intervals. The largest total amount given was 186 g in 31 days.

Gentisinamide showed a moderate reducing effect on temperature in the febrile cases; the effect was, however, not constant.

Pains were reduced in all cases, but only three of the patients were relieved from pain.

No unfavorable side reactions like nausea, tinnitus, dyspepsia or respiratory disturbances were noted. No signs of renal irritation were observed. In ten patients blood counts including hemoglobin levels were followed showing no significant alterations. Sedimentation rates did not change remarkably No depression of the prothrombine time was observed.

DISCUSSION

Clarke, Mosher & Clarke (1953) claim that a level of 3.5—5 mg of gentisic acid/100 ml plasma is necessary to suppress the manifestations of rheumatic fever. Assuming that gentisinamide exerts the same effect at the same concentration, 4.5 g has to be taken every three hours in the postabsorptive state to get an active blood level. Even a light meal depresses the plasma level in such a manner that the double dose would be necessary. The urinary excretion is so rapid that a cumulation is not probable. The clinical results also indicate that a curative blood level is not obtained. Perhaps more than 7.5 g of gentisinamide a day can be given. It is, however, not practical to supply a febrile patient with as much as 4.5 g every three hours and to have his stomach resting with so short intervals. Analgetic effect is often attained with lower doses than those needed in rheumatic fever. The clinical results, however, indicate that doses between 1 and 2.5 g three times a day do not prohibit pains abolished by equal doses of sodium salicylate or salicylamide.

SUMMARY AND CONCLUSIONS

An account of some chemical properties of gentisinamide is given.

The absorption of gentisinamide from the intestine seems to be rapid in the postabsorptive state but much delayed after a light meal. As the excretion of the compound is fast, active blood concentrations to combat rheumatic fever are not easily attained.

Gentisinamide combines, at least partially, with the albumins of plasma.

Gentisinamide in the dosage tried has a slight, not reliable antipyretic effect and is moderately analgetic, but definitely inferior to salicylamide or sodium salicylate.

Gentisinamide has not shown any toxic or other side-effects.

ACKNOWLEDGMENT

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THE PANCREATIC RESPONSE IN MAN TO THE INJECTION OF HIGHLY PURIFIED SECRETIN AND OF PANCREOZYMIN[1]

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A new method for preparing secretin, applying the ion exchange principle, has recently been developed by Jorpes & Mutt (1953). Since secretin has not been available for clinical use during the past few years, it was of considerable interest to ascertain whether this preparation could be used for the secretin test. We made ordinary secretin tests on 10 volunteers (healthy medical students) and, after the preparation had been found to be entirely free from toxic effects, on 6 infants as well. In three of the latter cases, the tests were combined with pancreozymin tests. This gave us an opportunity of studying the mutual participation of these two hormones in the stimulation of the pancreatic secretion in man.

Secretin

The secretin test was made after the experimental subjects had fasted for 5 hours. In the infants and in three of the adults, the duodenal juice was collected through a single lumen tube; in the other cases, an Ågren—Lagerlöf (1936) double-lumen tube was used. In the last-mentioned cases, the duodenal juice was allowed to flow spontaneously through the tube, whereas the gastric juice was aspirated with a syringe every 5 minutes. When a single lumen tube was used, the secretion was allowed to flow spontaneously; if the flow became interrupted, slight suction was applied or air injected into the tube.

The secretin used had been subjected to routine tests for sterility and freedom from pyrogens, and its activity determined in the cat. It was obtained as a freeze-dried powder in sterile vials containing 1 mg = 1.000 cat units. (1 cat unit is the quantity of secretin which yields 0.1 ml of 0.1 *N* alkali in 10 minutes after intravenous injection in the cat — Wilander & Ågren 1932.) This implies that with the usual dose of 1 clinical unit (= 16 cat units) per kg (Dreiling & Hollander 1948) it seemed unnecessary to give more than about 1 mg of this secretin to an adult.

Immediately before use, the secretin was dissolved in physiological saline solution (0.2 mg per ml); it was then injected intravenously at a fairly slow rate. No toxic reactions whatsoever were observed.

A definite flow of pancreatic juice was obtained with a first dose of 0.0025 mg per kg in two of the adults in whom a single-lumen tube was used. Fifteen minutes after the injection of secretin 16 and 17 ml of 0.1 *N* $NaHCO_3$ were obtained, as compared to 3 and 4 ml, respectively, before the injection. No tests were made with smaller doses.

[1] This investigation was aided by grants to Prof. E. Jorpes from the Magnus Bergvall Foundation, the Swedish Cancer Research Foundation and the Vitrum Ltd., Stockholm.

Table I. *Secretin test in 7 normal persons.*

Body weight	μg[1] secretin per kg	Duodenal juice, ml per 15 min. periods (every fig. corresponds to 15 min.)	Total volume duodenal juice, ml	Molarity of $NaHCO_3$ (every fig. corresponds to 15 min.)	Proteinase conc.[2]	Correlation between the secr. dose and the amount of $NaHCO_3$ secreted	
						secr. μg	ml 0.1 N alkali
kg	-	1.5	-	0.01	0.148	-	
	5	58—20—3	81	0.10—0.10—0.10	0.195	5	81 (in ¾ hour)
	10	79—30—16	125	0.11—0.09—0.08	0.200	10	127 »
kg	-	3	-	0.04	0.300	-	
	5	41—16—4	61	0.09—0.08—0.04	0.272	5	52 »
	15	69—50—17	136	0.11—0.13—0.14	0.123	15	165 »
kg	-	5	-	0.02	0.343	-	
	10	65—38—20—14	137	0.10—0.12—0.12—0.08	0.265	10	146 (in 1 hour)
	20	114—70—54—60	-	0.10—0.12—0.12—0.11	0.273	20	329 »
		—34—53	385	—0.10—0.05			
kg	-	14	-	0.01	0.155	-	
	7.5	50—20—10—40	120	0.10—0.08—0.03—0.01	0.268	7.5	73 »
	15	63—36—70—6	175	0.13—0.12—0.05—0.02	0.165	15	158 »
kg	-	10	-	0.0	0.340	-	
	7.5	40—33—24—6	103	0.11—0.13—0.10—0.05	0.258	7.5	114 »
	15	75—48—42—27	192	0.11—0.13—0.12—0.10	0.213	15	229 »
kg	-	3	-	0.0	0.283	-	
	5	29—5—0.5—0	34.5	0.12—0.11—0.10— —	0.170	5	42 »
	15	52—21—19—21	-	0.10—0.12—0.11—0.08	0.160	15	120 »
		—13	126	—0.04			
kg	-	5	-	0.01	-	-	
	10	53—46—30—10	139	0.09—0.12—0.08—0.07	-	10	134 »
	2.5	36—23—30—16	105	0.09—0.08—0.04—0.03	-	2.5	67 »

1 μg = 1 cat unit. 2 See footnote on page 231.

The results in the cases in which a double-men tube was used are recorded in Table I. It is seen from the table that two doses re given at an interval of 45 or 60 minutes, duodenal juice being collected in periods 15 minutes. In most of the cases, the dose of secretin was between 5 and 15 cat units or 0.005—0.015 mg of substance per kg of body weight.

A copious flow was obtained with a dose of 0.010 mg per kg. Double this dose was given on one occasion only; the quantity of 0.1 *N*

$NaHCO_3$ recovered in 80 minutes then amounted to 390 ml. No side-reactions occurred. We were able to confirm the observation of Hammarsten, Ågren & Lagerlöf (1937) that a large dose was not only associated with an increase in the output of pancreatic juice per time unit, but also with a marked prolongation of the duration of action of secretin.

It may be inferred from Table I that the dose (15 cat units per kg) most closely corresponding to that commonly used in the secretin test, *i. e.* 1 clinical unit (= 16 cat units) per kg, resulted in a total quantity of 120—229 ml of 0.1 *N* $NaHCO_3$ (1.9—3.3 ml per kg) or 113—192 ml of duodenal juice. The maximum concentration of bicarbonate varied between 0.120 and 0.140 millimols per ml.

No direct comparison can be made between these values and those given earlier in the literature, since details of the secretin test have varied with different workers. The figures nevertheless agree on the whole with earlier data. Dornberger, Comfort, Wollaeger & Power (1948), using 1 clin. unit of secretin per kg (Pancreotest, Astra, the Swedish secretin) obtained in 20 normal subjects in the course of 40 minutes after stimulation an average of 122.5 ml of duodenal juice of 0.105 normal alkalinity. Lake (1947), using a collection period of 60 minutes after the injection of 1 clin. unit per kg, obtained 62—242 ml of 0.1 *N* $NaHCO_3$. Diamond & Siegel (1940), Ågren, Lagerlöf & Berglund (1936) and Dreiling (1953) all used a collection period of 80 minutes after injection of 1 clin. unit per kg. In 24 cases Diamond & Siegel got 69—277 ml (1.0—3.9 per kg) of duodenal juice, the maximum concentration of bicarbonate being 0.050—0.130 millimols per ml. In 15 cases Ågren, Lagerlöf & Berglund got 2.5—5.0 ml of duodenal juice per kg body weight, the maximum concentration of $NaHCO_3$ being 0.094—0.137 millimols per ml. Dreiling got 99—449 ml (1.7—7.5 per kg) of duodenal juice using different secretin preparations including Pancreotest in a series of 91 normal subjects, maximum concentration of $NaHCO_3$ being 0.060—0.140 millimols per ml. In another series of 81 normal subjects he got 107—492 ml (1.8—7.6 per kg) of duodenal juice using the Wyeth secretin, the maximum concentration being 0.080—0.140 millimols per ml.

It is also evident from the table that, in the same individual, there was usually a good proportionality between the dose of secretin administered and the quantity of $NaHCO_3$ recovered at a given time after the injection. In two cases (3 and 7) the proportionality was, however, less marked; this must presumably be ascribed to the fact that the first dose was too large for its effect to have subsided before the next dose was given. This proportionality between the stimulating dose and the pancreatic response — pointed out earlier by Lagerlöf (1939) — is very striking.

To be considered in this connection are the rapidity of the response to the injection and the fairly large quantities of juice obtained with ordinary doses. It is presumably safest when injecting secretin always to start with a small dose — for example 5 cat units per kg — in order to ensure that there is a free flow of pancreatic juice. Otherwise, in the event of any obstruction of the pancreatic ducts, there might be a risk of injurious effects, such as edema of the pancreas. It has been shown in the dog that, when the pancreatic ducts are occluded, edema will develop within 3—4 minutes after the injection of secretin and will persist for several hours (Popper & Necheles 1942). When ischemia occurred under the same conditions necrosis also resulted; its degree of severity was parallel to the degree of edema at the

ble II. *Proteinase content of duodenal juice during repeated stimulation with secretin. The sleeping infants were given 0.020 mg secretin every 30 (case 1) or 15 (case 2) minutes.*

Case	Duodenal juice ml		$NaHCO_3$ molarity	Proteinase conc.[1]	total
Infant 1, 7.5 months old					
Before secretin stimulation	per 30 min.	6	0.02	0.730	4.4
During » »	»	11	0.06	0.751	8.3
	»	14.5	0.05	0.595	8.6
	»	20	0.08	0.435	8.7
	»	13.5	0.06	0.358	4.8
Infant 2, 11 days old					
Before secretin stimulation	per 15 min.	1	0.01	0.368	0.4
During » »	»	1.5	0.03	0.303	0.5
	»	2.5	0.05	0.368	0.9
	»	2.1	0.07	0.300	0.6
	»	3	0.07	0.315	0.9
	»	4	0.06	0.275	1.1
	»	2.2	0.06	0.253	0.6
	»	2.5	0.06	0.268	0.7
	»	2.2	0.07	0.240	0.5
	»	2.2	0.07	0.223	0.5
	»	2.2	0.05	0.220	0.5
	»	2.4	0.07	0.213	0.5

et of ischemia (Popper, Necheles & Rus- 1948). It may be questioned whether, in clinical discussion on various types of s of pancreatic function, sufficient regard been paid to results of this kind obtained n animal experiments.

The relation of secretin to the output of ymes was studied in a few infants during p, either spontaneous or induced by inistration of hypnotics. Only the pro- ase activity [1] was determined. Since the lt persons were awake, the nervous im- ses were subject to considerable variation in the course of the experiments. The enzymatic secretion of the pancreas is regulated by both a nervous and a hormonal mechanism, in contradistinction to the secretion of fluid and alkali, which is presumably conditioned mainly by hormonal stimuli (Babkin 1950). Consequently, a source of error arises in that the fluctuations in vagal tone are associated with a variation in the intensity of the secretory stimuli transmitted to the pancreas. The experimental conditions in the adults were therefore less suitable for the study of the influence of secretin on the

[1] The proteinase activity was determined according to Anson's hemoglobin method (1938) in the duodenal juice diluted 1 : 10. After the addition of Folin-Ciocalteus' phenol reagent to the trichloracetic acid filtrate of this method, the extinction was read off in a Beckman photometer at 750 mμ in a 10 mm cuvette. The value of E thus obtained was used as the direct expression of the proteinase activity. An expression of the relative value for the total quantity of proteinase was obtained by multiplying this value by the volume of duodenal juice.

output of enzymes by the pancreas. The irregular variations in the proteinase content of the duodenal juice are, in fact, evident from Table I.

In order to ensure that the effect of this source of error was as uniform as possible, the influence of secretin on the output of enzymes was studied during sleep instead; this could most easily be done in infants. The flow of pancreatic juice was then maintained by means of repeated injections of secretin, given every 15 or 30 minutes through a thin polyethylene catheter inserted percutaneously into the vein. As stated earlier, a single-lumen gastric tube was used in these cases; the duodenal juice was therefore somewhat diluted and neutralized by the gastric juice. However, judging by the values for the alkalinity of the duodenal juice, this source of error was fairly constant and therefore of no great importance.

In two infants sleeping spontaneously, a successively decreasing concentration of proteinase was noted after repeated large doses of secretin (Table II). The total enzyme content showed, however, only a slight tendency, if any, to decrease. In three cases in which the source of error implied by the variations in the depth of sleep was lessened by the administration of chloral or phenobarbital, a decrease in the concentration of the enzymes was recorded in connection with stimulation by secretin (Figs. 1, 2 and 3). After an initial increase, a fall was also noted in the total output of enzymes. In these cases, there was no diminution in the flow of pancreatic juice, since secretin was injected every 15 or 30 minutes; this is presumably essential if any conclusions are to be drawn from a decrease

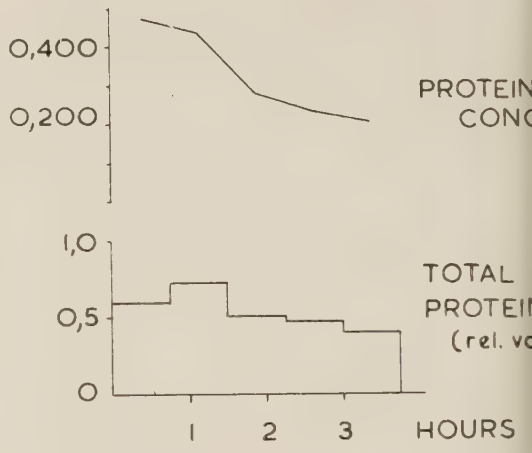

Fig. 1. Proteinase content of the duodenal juice during stimulation with secretin. Infant 17 days old (diagn. Mongolism), sleeping on phenobarbital. 0.020 mg secretin every 15 min.

in the total output of enzymes during stimulation with secretin. Thus, the secretin preparation in question had no stimulating effect on the secretion of enzymes.

Secretin combined with pancreozymin

In order to ascertain the order of magnitude of the output of enzymes during stimulation with secretion in relation to the actual functional ability of the pancreas, tests were made in three cases with secretin in combination with pancreozymin. A small quantity of pancreozymin prepared by Jorpes and Mutt was obtained in a sufficiently pure state for clinical use. Pancreozymin was tested in man by Duncan, Harper, Howat, Oleesky & Varley in 1950. They found that similar to the conditions in the cat, its administration resulted in an increase in the output of enzymes; they did not, however, state the order of magnitude of this increase. Howat (1952) gave an account of some

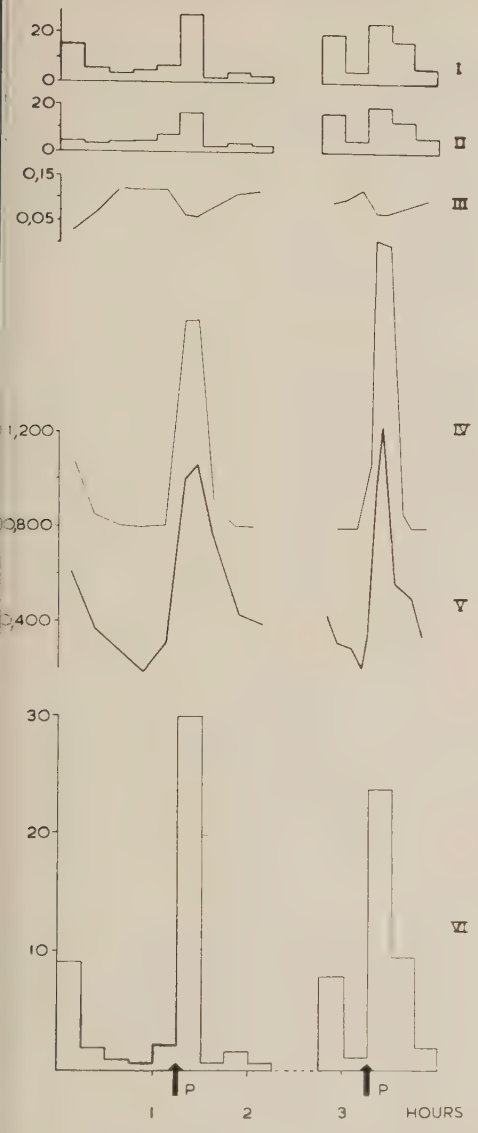

Fig. 2.

2. Proteinase content of duodenal juice during ulation with secretin. Infant 1 year old (diagn. drocephalus), sleeping on chloral. 0.010 mg etin per kg every 15 min., pancreozymin 0.5 mg kg given at."P".(---- = interruption for 30 min. secretin given and duodenal juice not collected.)
Volume of duodenal juice, ml.
Volume of 0.1 *N* alkali, ml.
Molarity of alkali.

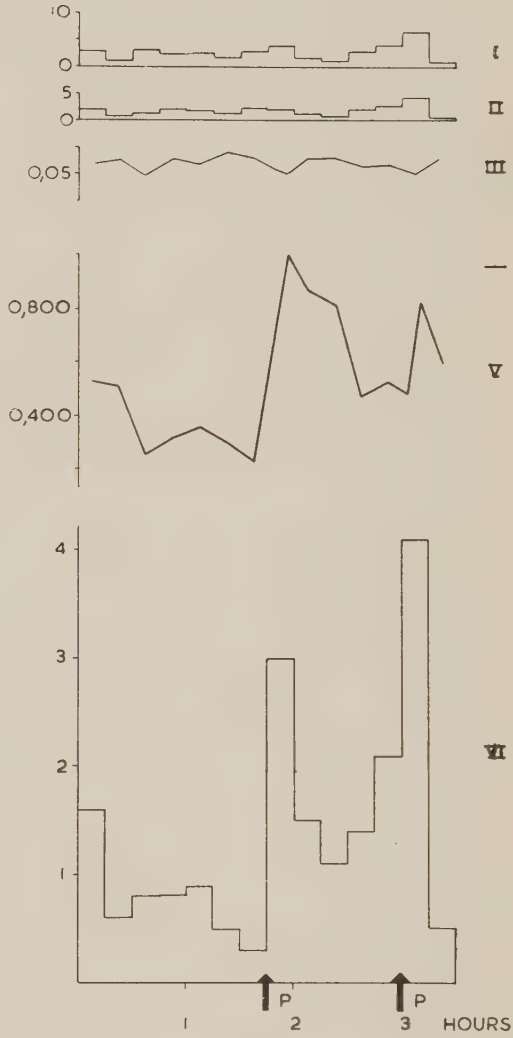

Fig. 3.

IV. Icterus index.
V. Proteinase-concentration of duodenal juice.
VI. Proteinase total values.

Fig. 3. Proteinase content of the duodenal juice during stimulation with secretin. Infant 3 months old (diagn. Hydrocephalus), sleeping on chloral. 0.010 mg secretin per kg every 15 min.; pancreozymin 0.5 mg per kg given at "P".
I—VI: see Fig. 2.

clinical secretin-pancreozymin tests carried out on normal subjects and on subjects suffering from pancreatic disease. He injected secretin only once before injecting pancreozymin. The secretin-induced juice was consequently rich in enzymes due to the washing out effect of secretin. The output of enzymes after the injection of pancreozymin was only moderately increased as compared with the output after secretin.

We have, before injecting pancreozymin in our cases, induced a continuous flow of pancreatic juice by stimulation with secretin for at least one hour. Thereby we were able to compare the effects of secretin and pancreozymin after the initial washing out period.

In two of our cases (Figs. 2 and 3), repeated injections of secretin were first given in the course of at least one hour; this was followed by an injection of pancreozymin (0.5 mg per kg). Another secretin period followed, after which another injection of pancreozymin was given. In the third case (Fig. 4) secretin was continuously injected and pancreozymin given only once.

During the initial secretin period, the proteinase concentration and the total output of enzymes fell to a certain minimum level. This was succeeded by a considerable rise both in the total quantity of proteinase and of its concentration in the duodenal juice after the first injection of pancreozymin. In the two cases with a subsequent secretin period a fall was once more noted, the second injection of pancreozymin again resulting in a marked rise in the output of enzymes.

In two cases (Figs. 3 and 4) there was a slight increase, and in one (Fig. 2) a considerable increase, in the volume of duodenal juice after the injection of pancreozymin,

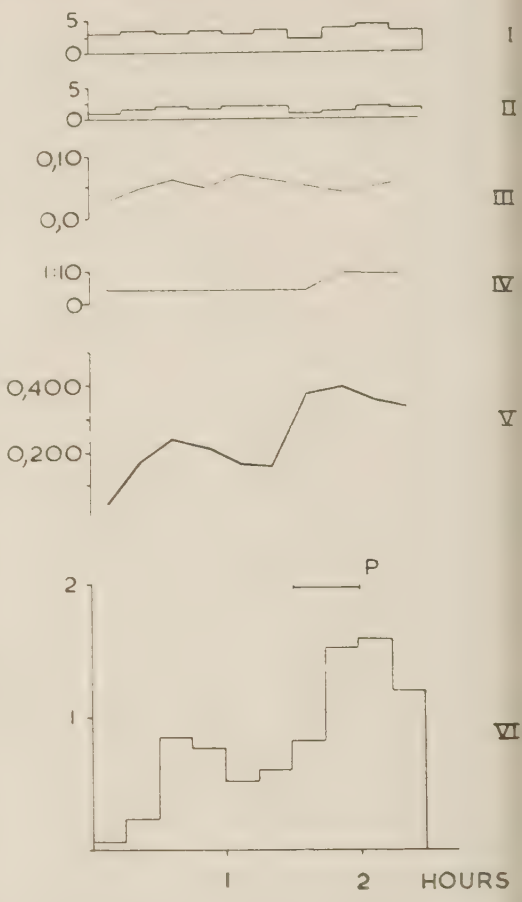

Fig. 4. Proteinase content of duodenal juice during continuous stimulation with secretion injected at a rate of 0.030 mg per kg and 15 min.; pancreozymin 0.5 mg kg at "P". Infant 13 days old (diagn. Mongolism), sleeping on chloral. I—VI: see Fig. 2

mainly owing to the contraction of the gall-bladder associated with the injection. The admixture of bile is evident from the icterus index curve for the duodenal juice shown in Fig. 2. Despite this dilution, the enzyme concentration rose appreciably. In all three cases, the maximum values were actually higher than may be inferred from the figures. This is because the enzyme concentration o

duodenal juice gave a color intensity
n fell outside the range of proportionality
veen the color and the quantity of sub-
ce. The ratio of the total quantity of
einase excreted during the 15 minutes
ediately before the injection of pancreo-
in to that during the 15 minutes imme-
ely after it was in the case related to
2, 2:29 in the first and 1:24 in the
nd test.

onsequently, in view of the appreciable
ease in the output of enzymes after
ulation with pancreozymin, no definite
ements can be made regarding the total
me-producing capacity of the pancreas
he basis of the pancreatic response to
ctions of secretin alone.

SUMMARY

A highly active secretin preparation has
n tested clinically. A definite pancreatic
onse was obtained with the intravenous injection of 0.0025 mg of secretin per kg of body weight.

The response to a dose of 0.015 mg per kg was of the order of magnitude generally obtained in the secretin test in man reported in the literature.

There was a stoichiometric proportionality between the magnitude of the dose of secretin injected and the quantity of 0.1 *N* bicarbonate secreted in the duodenal juice.

The effect of repeated injections of secretin was studied in sleeping infants. It is concluded that secretin has no stimulating action on the enzyme secretion.

In three infants in whom a continuous flow of pancreatic juice was maintained by continuous or repeated stimulation with secretin, pancreozymin was injected. This was found to increase the output of enzymes up to 20 times during the period immediately following the injection, as compared to the output during a similar period before the injection of pancreozymin.

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METHOD FOR QUANTITATIVE DETERMINATION OF PEPSIN IN GASTRIC JUICE

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n the determination of pepsin activity rthrop (1932) used as substrate the non-stallisable casein "Hammarsten". He re-rked, however, that different prepara-s, even if carefully produced on the basis he same prescriptions, may give substrate tions showing considerable (mutual) iations in the rate of proteolysis with the e amount of pepsin. As commercials of k powder *e. g.* "Klim" were very accu-ly reproduced products in this respect, rthrop proposed to use them as substrates experimental proteolysis. On the basis his fact Ege & Menck-Thygesen (1933) porated a method for pepsin determination h milk powder solutions as substrate. rrestrup-Andersen & Bock (1948) as well Hunt (1948) later estimated the pepsin vity of gastric juice by means of spray ed serum protein as substrate. In the sent paper the applicability of this sub-te is further investigated.

PRINCIPLE OF THE METHOD

he proteolytic process proceeds in a sub-te solution prepared from spray-dried an serum protein. The process is inter-ted by precipitation with trichloracetic , after which the concentration of the ble protein split products in the filtrate, which is used as a measure for the amount present of active proteinase, is determined by means of Folin-Ciocalteu's reagent. Using a reference enzyme we are able to express the pepsin content of gastric juice in terms of mg of standard pepsin as described below.

REAGENTS, METHOD AND STANDARD CONDITIONS

Spray-dried human serum protein is prepared by *The State Serum Institute,* Copenhagen, according to the method described by Marcussen (1945). According to the investigations of Bock (1949) the prepared substance has a rather constant protein concentration, and the relative albumin percentage of liquid serum is not influenced by the process of drying. This is in accordance with Terp (1953) who found that only negligible amounts of procain esterase was destroyed in the atomizer. Spray-dried serum from *The State Serum Institute* is dispensed in sealed bottles containing about 40 g serum protein powder from 50 to 150 persons. The contents of 30 to 40 bottles are thoroughly mixed and divided in 3 or 4 portions, which are stored in the refrigerator in carefully stoppered bottles.

Sørensen's 0.1 M glycocoll buffer solution is composed as follows: 45 ml of 0.1 N

Trichlor-acetic acid	②	④	Substrate	Trichlor-acetic acid	⑥	⑧	Substrate	. . ect.
Substrate	①	③	Trichlor-acetic acid	Substrate	⑤	⑦	Trichlor-acetic acid	. . . ect.

Fig. 1. Arrangement of substrate and trichloracetic acid tubes for proteolytic tests.

hydrochloric acid plus 55 ml of 0.1 M glycocoll/sodium chloride solution. The pH of this solution is 2.10, as controlled by glass electrode.

Strongly acid buffer solution for adjustment of the pH of the substrate solution is composed as follows: 45 ml of 1.0 N hydrochloric acid plus 100 ml of the described Sørensen's 0.1 M glycocoll buffer solution.

Trichloracetic acid 0.350 N adjusted by titration with phenolphthalein as indicator (Hunt, 1948).

Sodium hydroxide 0.250 N adjusted by titration with phenolphthalein as indicator (Hunt, 1948).

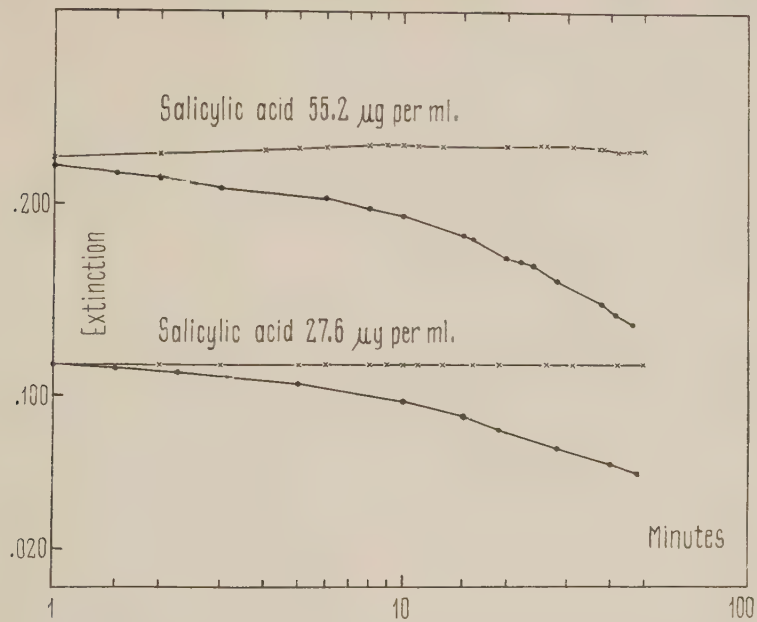
As reference enzyme was used *Pepsin 1: 10 000 General Biochemical Industries* from a large thoroughly mixed quantity, which was stored in ampoules of 0.5 g, sealed in nitrogen atmosphere, after drying over sulphuric acid.

Procedure

10 g serum protein powder was dissolved in 50 ml of water in a 100 ml graduated flask, under vigorous shaking. Next 38.2 ml of the strongly acid buffer solution were slowly added under continuous stirring to avoid precipitation of protein. Now 1 or 2 drops of octyl alcohol were added, and the flask was filled up to the mark. pH of the substrate solution was 2.10, as controlled by the glass electrode. Measurement of the pH before and after the course of the protelytic process showed the buffer capacity of the system to be adequate.

Pairs of test tubes, one of which contained 5 ml of substrate solution plus 1 ml of Sørensen's buffer solution and the other 10 ml of trichloracetic acid were placed in a metal rack together with the pepsin solutions to be examined for 10 minutes pre-heating on a water bath at 37° C. Each pepsin determination comprised four tubes, as shown in Fig. 1.

After pre-heating 1 ml of pepsin solution was added to substrate tube 1, under shaking to insure thorough mixing of enzyme and substrate. 30 seconds later 1 ml of the same enzyme solution was added to trichloracetic acid tube 3. After another 30 seconds 1 ml of the next enzyme solution was added to substrate tube 5, and again 30 seconds later 1 ml of this to trichloracetic acid tube 7, etc. The enzyme solutions were, thus, added alternately to the substrate tubes and the trichloracetic acid tubes with the odd numbers. The times were taken with a stop clock. After 15 minutes of reaction the solutions in tubes 1 and 2 were mixed by pouring them several times from tube to tube. This caused instantaneous cessation of the proteolytic process and precipitation of the non-digested protein. 30 seconds later the solutions in tubes 3 and 4 were mixed, and so forth through the whole series. The mixing was always carried out at intervals of 30 seconds to maintain a reaction time of



2. Extinction in Beckman's spectrophotometer at 740 mµ when 1 ml salicylic acid solution plus ml destilled water (•) or 1 ml salicylic acid solution plus 1 ml trichloracetic acid filtrate (x) uce 1 ml Folin-Ciocalteu's reagent in 20 ml 0.250 N sodium hydroxide as a function of time. 1.000 cm adjusted glass absorption cells.

minutes. Apart from the proteolytic lt, the mixed contents from tubes 1 2 were identical with those from tubes d 4. Filtration through Munktell's filter er No. 00M gave a limpid, colorless fluid, ch was used for the colorimetric analysis. s was performed as follows: 1 ml of the ate was pipetted into 25 ml of 0.250 N um hydroxide in an Erlenmeyer flask. ediately after, 1 ml of Folin-Ciocalteu's ent was carefully pipetted down under surface. The contents of the flask were mixed by swinging. 1 ml of the corre-ding blank was treated in the same way. inutes after the mixing of the reagents ifference in extinction was read in Beck-'s spectrophotometer at 740 mµ using 1.000 cm adjusted glass absorption cells or in a colorimeter fit for the purpose, *e. g.* Unicam junior.

Control of Folin-Ciocalteu's reagent

In the presence of phenols and at strong alkaline reaction, Folin-Ciocalteu's reagent develops a blue color owing to a reduction of the molybdenum and tungsten compounds of the reagent. Various Folin-Ciocalteu reagents must, however, be expected to differ with regard to the development of the blue color. It is therefore necessary to dispose of a chemically well-defined substance, which can act as primary substance of Folin-Ciocalteu's reagent. Several authors have controlled the reagent with standard solutions

of tyrosine (Anson & Mirsky, 1932) or standard solutions of phenol (Hunt, 1948). Tyrosine is, however, difficult to produce and slightly soluble, and phenol is, according to the author's experience, too sensitive to oxygen or impurities to be of practical value. We have therefore preferred the phenol salicylic acid (Acidum salicylicum Ph. Dan. 1948) as primary substance of Folin-Ciocalteu's reagent. Salicylic acid is a stable substance, readily available and has a relatively high solubility. By adding 1 ml of aqueous salicylic acid solution and 1 ml Folin-Ciocalteu's reagent to 20 ml 0.250 N sodium hydroxide the blue color produced was unstable (Fig. 2). But by adding 1 ml trichloracetic acid filtrate both for the salicylic acid-sodium hydroxide solution and the blank sodium hydroxide solution the color intensities remained unchanged for about 50 minutes, then faded steadily. We must suppose that the trichloracetic acid filtrate, in addition to phenol groups which reduce the reagent, contain substances that stabilise the color when the reduction has taken place. The salicylic acid curve in Fig. 3 was plotted on this principle. In this way the strength of Folin-Ciocalteu's reagent was easily controlled and compared with reagents prepared at different times. Folin-Ciocalteu's reagent was always prepared by the author himself according to the original prescription (1927). As mutual deviations between several reagents never exceeded 2 to 3 per cent, no correction was introduced for these deviations. The individual reagent was controlled every 3 or 4 months by means of standard salicylic acid solutions freshly prepared for that purpose. This control showed that the reagent when stored at 0° C in

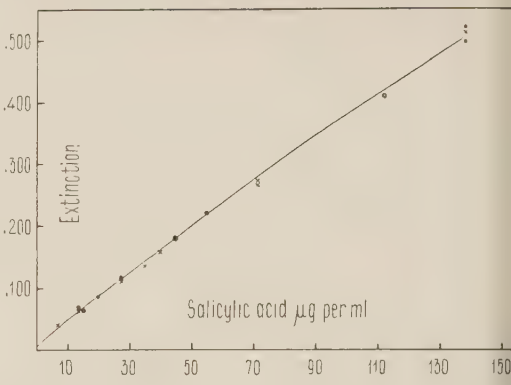
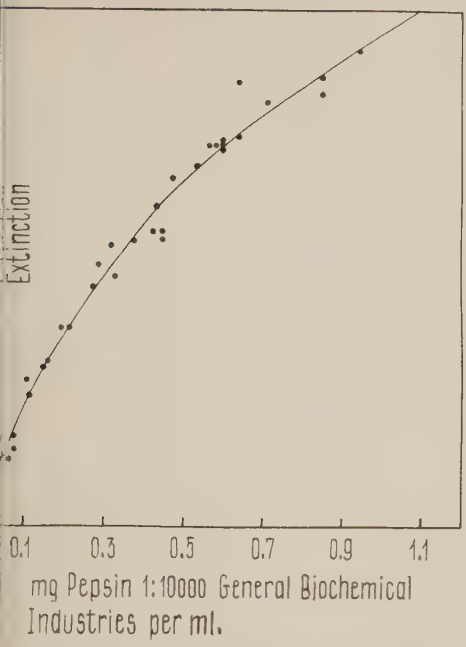


Fig. 3. Relation between extinction in Beckman' spectrophotometer at 740 mµ when 1 ml salicyli acid solution plus 1 ml trichloracetic acid filtrat reduce 1 ml Folin-Ciocalteu's reagent in 20 m 0.250 N sodium hydroxide and concentration o salicylic acid (µg) per ml.

bottles (alkaline-free glass) with glass stoppers was stable for at least 6 months with regard to the stated color-producing property. It was never necessary to regenerate a Folin-Ciocalteu's reagent once prepared

Technical details

Experiments have shown that variation of the substrate concentration between 9 and 11 g serum protein per 100 ml had no influence on the proteolytic result. A centigram balance weighing was therefore sufficiently accurate. Further, it has been found that repeated proteolysis of the same substrate with equal amounts of pepsin gave identical results within 14 days, provided the substrate solutions were stored in the refrigerator at 0° C. Kept at room temperature, however, the substrate was unfit for proteolytic tests within 24 hours owing to a rising blank value. Experiments have shown that repeated colorimetric analysis o



. Relation between extinction in Beckman's rophotometer at 740 mμ and concentration of n 1 : 10000 General Biochemical Industries in er ml after 15 minutes of proteolysis at 37° C.

richloracetic acid filtrates remained unged within 14 days provided they were in stoppered tubes at 0° C. Standing oom temperature, on the other hand, ined a rise of the colorimetric extinctions dy after 12 hours. These facts were of rkable value for the applicability of the od in practice, especially when many eolytic experiments were performed on same day. Turbidity or fine precipitate l occasionally be seen in the trichlorc acid filtrates kept for some time in the gerator. This applied exclusively to triracetic acid filtrates of reaction mixtures ct to considerably cleavage. If, howthese filtrates were thoroughly shaken e being pipetted off for the colorimetric sis, this was not influenced.

The reference enzyme

Fig. 4 illustrates the activities of Pepsin 1 : 10000 General Biochemical Industries under the stated experimental conditions. The enzyme was dissolved in Sørensen's 0.1 M buffer solution. With this enzyme preparation it has always been possible to check the digestibility of the substrate. In other words it acts as a primary substance of the substrate, like salicylic acid acts as a primary substance of Folin-Ciocalteu's reagent. It may be seen from Fig. 4 that the concentration of 0.15 mg per ml corresponds to the extinction of 0.200 on the ordinate.

DETERMINATION OF PEPSIN IN GASTRIC JUICE

The method described proved useful for determining the pepsin concentration in gastric juice. After filtration, by which mucin particles, minor coagles, *etc.* were removed, the limpid gastric juice was diluted five to ten-fold with Sørensen's buffer solution. The pH was thereby fixed at 2.10. The procedure of analysis was otherwise exactly the same as previously described for solutions of the reference enzyme.

Fig. 5 shows the results of analyses of four samples of gastric juice in different dilutions. It may be seen that we found no proportionality between the concentration of the gastric juice and the cleavage expressed by the colorimetric extinction of the trichloracetic acid filtrates. To be able to determine the pepsin activity of the gastric juice independently of the degree of dilution it was therefore necessary to define a standard or unit. Most writers employ an arbitrary system of units. A common procedure is that of defining the extinction of a standard

color as representing 50 pepsin units, for instance. The percentile concentration of the gastric juice that produces this extinction under the experimental conditions employed, therefore, by definition contains 50 pepsin units (Anson & Mirsky, 1932, Anson, 1938, Hunt, 1948).

Like Ege & Menck-Thygesen (1933) and Harrestrup-Andersen & Bock (1948) we have preferred a more concrete indication of the pepsin concentration of the gastric contents, namely in milligram per millilitre. Pepsin 1 : 10000 *General Biochemical Industries* was used for that purpose. It would, of course, have been most correct to use a pepsin preparation of human origin, but this is not available.

In the present paper we have preferred to define the gastric juice to contain 0.15 mg Standard Pepsin 1 : 10000 per millilitre in the percentile concentration that in the described system produces a cleavage of the substrate corresponding to a colorimetric extinction of 0.200 in Beckman's spectrophotometer at the wavelength 740 mμ using 1.000 cm glass absorption cells. It appears from Fig. 5 that an 8 per cent concentration of the gastric juice 1289/52 produced a cleavage of the substrate corresponding to the extinction 0.200 indicating that this concentration contained 0.15 mg Standard Pepsin 1 : 10000 per ml. In other words it had exactly the same proteolytic activity as if it contained 0.15 mg Pepsin 1 : 10000 General Biochemical Industries per ml. The concentration of 20 per cent gave a cleavage corresponding to 0.375 and therefore contained $20 \times 0.15/8 = 0.38$ mg Standard Pepsin 1 : 10000 per ml. Concentration of 12 per cent of gastric juice 7/4 52, 20 per cent of 4698/52 and undiluted gastric juice 1104/52

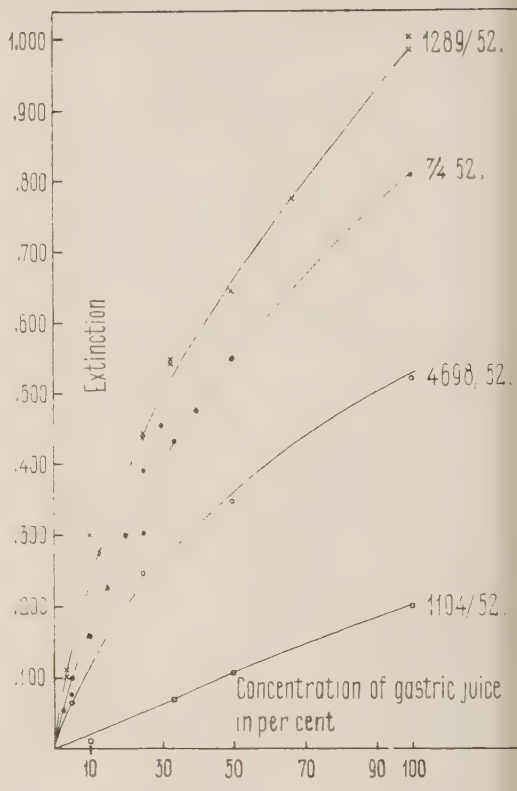
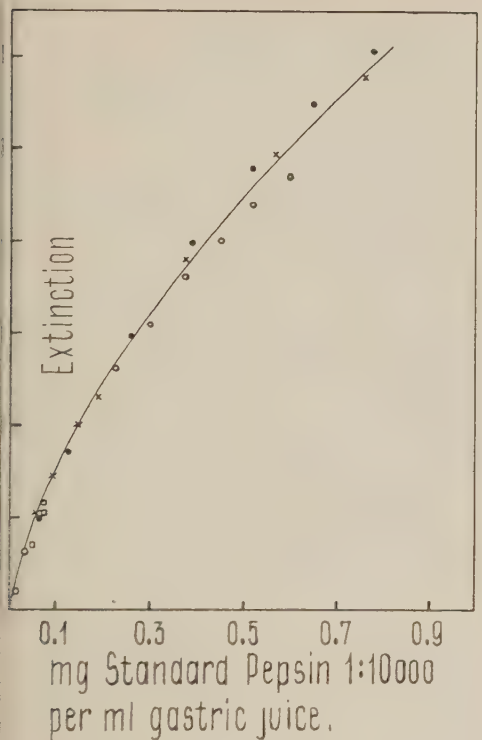


Fig. 5. Influence of dilution of gastric juice on th proteolytic result. Proteolysis with four sampl of human gastric juice.

all produced a cleavage corresponding to a extinction of 0.200, which, according to ou definition, indicated a concentration of 0.1 mg Standard Pepsin 1 : 10000 per ml. By th described procedure it is therefore possib to express the concentration of Standar Pepsin in mg per ml of any sample of gastr juice when the dilution is known. The curv of Fig. 6 was plotted on this principle. It very similar to that of Fig. 4, with whic it has the point 0.200 on the ordinate an 0.15 on the abscissa in common. If supe imposed it may be seen that the differen between them corresponds to the analyt



6. Relation between extinction in Beckman's trophotometer at 740 mμ and concentration of ndard Pepsin 1:10000 in mg per ml human stric juice. 1.000 cm glass absorption cells.

or when working with extinctions lower n about 0.450.

Table I illustrates the concentration of ndard Pepsin 1: 10000 in mg per ml nan gastric juice according to the author's lysis and according to the analysis of ther investigator in the same laboratory. nay be seen that the error of the described hod is between + 9.5 and —8.6 per cent.

SUMMARY

Our investigations have shown that spray-d human serum protein from *The State um Institute,* Copenhagen, is a useful substrate for determining the peptic activity of gastric juice as originally suggested by Harrestrup-Andersen & Bock (1948) and Hunt (1948).

The pepsin concentration of gastric juice is expressed in milligram Standard Pepsin 1: 10000 per millilitre, a more concrete indication than the frequently used systems of arbitrary pepsin units.

To check the digestibility of the substrate we use a commercial enzyme preparation stored at —21° C in ampoules sealed in nitrogen atmosphere after drying over sulphuric acid.

The peptic cleavage of the substrate is measured in Beckman's spectrophotometer under standardised experimental conditions. Using standard solutions of salicylic acid as primary substance of Folin-Ciocalteu's rea-

Table I. *Standard Pepsin 1:10000 in mg per ml gastric juice determined by two investigators.*

Standard Pepsin 1: 10000 in milligram per millilitre gastric juice

Sample no.	According to the author	According to another investigator	Deviation in mg per ml	Deviation in per cent
1	1.6	1.7	+ 0.1	+ 6.2
2	2.7	2.9	+ 0.2	+ 7.4
3	1.5	1.6	+ 0.1	+ 6.7
4	2.2	2.3	+ 0.1	+ 4.5
5	1.3	1.3	—	—
6	2.7	2.8	+ 0.1	+ 3.7
7	2.3	2.1	— 0.2	— 8.6
8	2.2	2.2	—	—
9	1.8	1.7	— 0.1	— 5.6
10	2.1	2.3	+ 0.2	+ 9.6
11	1.7	1.7	—	—
12	1.3	1.4	+ 0.1	+ 7.8
13	0.0	0.1	—	—
14	1.3	1.4	+ 0.1	+ 7.8

gent it has been found that its color-developing strength remains unchanged for months, and that there are only negligible differences between various reagents. By adding small amounts of trichloracetic acid filtrate to the alkaline reaction mixtures before the addition of Folin-Ciocalteu's reagent, the colorimetric extinction of the developed salicylic acid color is stabilized for about 50 minutes. If not, the color will fade rapidly.

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THE EFFECT OF SERA FROM SCHIZOPHRENIC ATIENTS ON GLUCOSE UTILIZATION OF THE ISOLATED RAT DIAPHRAGM

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Disturbances in carbohydrates metabolism he insane have often been observed. Thus, earlier reported by one of the authors, nuria (Lingjærde, 1934) and patho- cal insulin tolerance test (Lingjærde, 3) frequently occur in schizophrenia. In tinuation of these studies investigations possible hormonal disorders in schizo- enic patients are being carried out at r Mental Hospital.

n the present paper the problem has been estigated by studying the effect of sera n schizophrenics on glucose utilization the isolated rat diaphragm.

Theoretically the work is based on the ervation that carbohydrate metabolism of isolated diaphragm is affected by hor- nes *in vitro*. As first shown conclusively Gemmill (1941) and since by many ers, addition of insulin to the incubation dium stimulates glucose uptake and sub- uently glycogen synthesis of the dia- agm. Other hormones act antagonisti- y. Thus, pituitary growth hormone rahl, 1951, Park, 1952), adrenal cortical roids of the C-11-oxy type (Grossman, der & Pearson, 1953) and epinephrine Walaas & Walaas, 1950) decrease glucose ization of the diaphragm.

A stimulating effect of human blood serum glycogen synthesis of the diaphragm was observed by Tuerkischer & Wertheimer, (1948) and Perlmutter & Greep (1948). Further Groen, Kamminga, Willebrands & Blichman (1952) reported stimulation of glucose uptake of the diaphragm by normal human serum. The same effect was found by Park (1952) using normal rat serum. The nature of this serum effect is not fully understood and needs further investigation. Howewer, as will be mentioned later, there is strong evidence that hormonal factors are of major significance.

In the present work it has been shown that sera from schizophrenics exert a decreased stimulation of glucose uptake of the diaphragm compared with the effect of sera from normals.

METHODS

Young male rats weighing 120—130 g were used for the experiments. The rats were starved 24 hours before experiment. The diaphragms were removed and incubated in Krebs-Ringer medium, containing 0.01 M phosphate buffer pH: 7.4. Incubation was made in 100 per cent oxygen at 37° C for 1 hour in a Warburg apparatus. The procedure was similar to that reported by Krahl & Park (1948). One hemidiaphragm was used as control and incubated in 2 ml Krebs-Ringer medium. The other

Table I. *Effect of normal sera on glucose utilization of rat diaphragm.*

Experimental person	Glucose utilization mg per g wet tissue per hour		
	Control	0.4 ml serum added	Increase
1.	2.4	3.7	1.3
2.	3.1	6.1	3.0
3.	2.9	5.3	2.4
4.	2.3	3.0	0.7
5.	1.4	2.7	1.3
6.	1.8	3.0	1.2
7.	2.0	3.1	1.1
8.	2.4	3.9	1.5
9.	2.6	3.7	1.1
Mean	2.3 ± 0.18 [1]	3.8 ± 0.38	1.5 ± 0.24

[1] Standard error.

Procedure: Krebs-Ringer phosphate medium pH: 7.4. Glucose concentration adjusted to 140 mg per cent in all experiments. Incubation in 100 per cent O_2 at 37° C for 1 hour.

Statistical test on the significance of difference in glucose uptake.

t: 3.6, D.F.: 16, P: 0.003.

Table II. *Effect of sera from schizophrenics on glucose utilization of rat diaphragm.*

Experimental person	Glucose uptake mg per g wet tissue per hour		
	Control	0.4 ml serum added	Increase
S.M.	1.2	2.1	0.9
S.M.	2.2	3.4	1.2
S.M.	2.1	2.6	0.5
O.H.	3.8	4.1	0.3
O.H.	2.6	3.0	0.4
A.E.	1.8	2.7	0.9
Mean	2.3 ± 0.36	3.0 ± 0.28	0.7 ± 0.14

Procedure: The same as described in Table I.

Patient S. M.: ♀ 29 years old, schizophrenic for 15 years. Leukotomy 3 years ago without effect.

Patient O.H: ♀ 36 years old. Schizophrenic for 2 years. Classical symtoms. Leukotomy 1 year ago without effect.

Patient A.E.: ♀ 46 years old. Schizophrenic for 3 years. Classical symptoms.

Statistical test on the significance of increase in glucose uptake, normal individuals versus schizophrenics.

t: 2.5, D.F.: 13, P: 0.03.

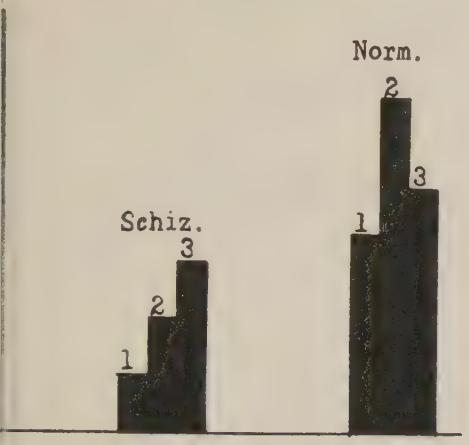
hemidiaphragm was incubated in 1.6 ml Krebs-Ringer + 0.4 ml serum. The initial concentration of glucose was adjusted to 140 mg/100 ml in both vessels. 3 hemidiaphragms of a total weight of 200 mg muscle tissue were added in each vessel. Glucose utilization of the diaphragm was determined by measuring the glucose concentration of the incubation medium before and after experiment. The method of Nelson (1944) was used for glucose determinations. The values are expressed in mg glucose per g wet diaphragm per hour.

Samples of sera were taken from normal healthy individuals in the hospital and from psychiatric patients under the same conditions. Blood samples were taken at 8. a. m. under fasting conditions. The cells were centrifuged down immediately and serum used in experiment within 2 hours. Where no other information is given the experimental persons had lived on the same general diet.

RESULTS

In Table I the effect of 0.4 ml normal serum on glucose uptake of rat diaphragm is shown. In all experiments a stimulation occurs, averaging 1.5 mg glucose per gram per hour, or 65 per cent of the mean control value, a statistically significant increase ($P = 0.003$).

In Table II experiments on sera from 3 patients with schizophrenia in an active phase are shown. A brief history of the disease is



1. Stimulation of glucose uptake (mg per g our) of rat diaphragm by serum from a normal a schizophrenic person (A. E). Different nts of serum were added. 1: 0.3 ml serum. 2: 0.4 ml serum. 3: 0.5 ml serum.
edure: The same as described in Table I. The amount of incubation medium was 2.0 ml and se concentration adjusted to 140 mg per cent in all vessels.

rted in the table. In this group the ulation of glucose uptake by addition of nl serum was less than observed for sera ormal individuals. The average stimula- from 6 experiments was 0.7 mg per n per hour or 30 per cent of the controls. istically the difference in stimulation of ose uptake in Table I and Table II was robable significance ($P = 0.03$).

he effect of different amounts of serum ed to the incubation mixture has also studied (Fig. 1). In these experi- ts the vessels always contained a total 0 ml incubation medium. 0.2 ml serum from normal and schizophrenic persons without any significant effect. On in- sing the amount of serum from 0.3 ml .5 ml, increased stimulation of glucose utilization was observed. Maximal stimulation was obtained by adding 0.4 ml to 0.5 ml normal serum. As shown in the diagram the stimulation was less with serum from the schizophrenic at the different doses used.

Table III. *Effect of sera from a schizophrenic treated with insulincoma (according to Sakel) on glucose utilization of rat diaphragm.*

Experimental person	Glucose utilization mg per g wet tissue per hour		
	Control	0.4 ml serum added	Increase
P.N.	2.4	3.4	1.0
P.N.	2.0	3.4	1.4
P.N.	1.8	3.6	1.8
P.N.	2.0	3.4	1.4
Mean	2.0	3.4	1.4

Procedure: The same as described in Table I.

Finally a schizophrenic patient was studied who had been treated with insulin-coma ad modum Sakel 3 months earlier with temporary improvement. In 4 experiments serum from this patient produced stimulation of glucose utilization of the order of magnitude observed with sera from normals (Table III). Thus the mean value of stimulation was 1.4 mg glucose per gram per hour.

DISCUSSION

The present investigation has confirmed earlier observations in showing a stimulation of glucose utilization of the isolated rat diaphragm by addition of normal human serum to the incubation medium. The new observation which has been made is that in sera from schizophrenics an inhibition of the serum effect occurred.

The effect observed in normal serum can either be due to non-hormonal or hormonal substances present in serum. Park (1952) reported a slight stimulation of glucose uptake of the diaphragm by addition of 3 per cent bovine serum albumin to the medium. A much stronger stimulation was obtained with normal rat serum.

Groen *et al.* have brought evidence that the stimulating effect of human sera is due to the presence of insulin. Thus, the effect was absent in sera from patients in diabetic coma and also in sera of pancreatized dogs. Further, the stimulation by normal sera disappeared when glutathione was added.

On the other hand experiments made by Bornstein & Park (1953) indicate that pituitary and adrenal substances are of major importance. In sera from alloxan diabetic rats a stimulating effect was lacking. However, in these animals hypophysectomy or adrenalectomy restored the stimulation of glucose uptake by serum. It was therefore supposed that the inhibition of the serum effect in sera from alloxan diabetic rats was due to the presence of substances released from pituitary and adrenal cortical glands. By addition of insulin *in vitro* the inhibitory effect of serum from diabetic rats was reversed. Bornstein (1953) has further shown that inhibition of glucose uptake of diaphragm is exerted by a lipoprotein fraction of serum from alloxan diabetic rats. This inhibitory compound is not present in lipoprotein fractions of sera from normal rats.

Therefore, at the present stage of knowledge, it seems permissible to conclude that hormonal factors are essential for the stimulation of glucose uptake of rat diaphragm by serum.

The observation mentioned may indicat the assumption that the decreased stimu lating effect in sera from schizophrenics ma be due to altered hormonal factors in serun Of course, nonhormonal factors must also b taken into consideration. Disorders in carbo hydrate metabolism, such as ketonuria an pathological insulin tolerance test were simul taneously observed in the patients studied. I is, however, unlikely that increased aceto acetate concentration in serum is of im portance, as Groen *et al.* did not find an effect of this compound on glucose uptak of the diaphragm.

The hypothesis may be advanced that in hibition of the serum effect in schizophrenic is due to substances of pituitary or adrena origin. However, further experiments ar needed to test this assumption. It seems c interest in this connection that signs of in creased adrenal cortical function are ofte observed in active phases of schizophreni (Lingjärde, 1953).

In the patient who had been treated wit insulin-coma *a. m.* Sakel the serum effec was normal. It should be mentioned tha signs of disorders in carbohydrate meta bolism in this patient had disappeared afte the treatment. Thus, the insulin toleranc test was normalized and ketonuria wa absent.

Further work is in progress to characteriz the inhibition of the serum effect in schizo phrenia reported in this preliminary study

SUMMARY

1) Normal human blood serum stimulate glucose utilization of the isolated r diaphragm.

Stimulation of glucose uptake of the diaphragm by sera from 3 schizophrenic patients is less than that found in normal sera. In these patients disorders in carbohydrate metabolism were observed. Serum from a schizophrenic patient who had been treated with insulin-coma *a. m.* Sakel, stimulated glucose uptake of diaphragm at the normal order of magnitude. In this patient disorders in carbohydrate metabolism were normalized after the treatment.

The observations made have been discussed in relation to recent knowledge on the hormonal nature of the serum effect.

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A NEW NITROGEN METER MODEL

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During recent years the Lilly Nitrogen meter (Lilly, Andersson & Howey 1943) has been widely used in respiratory research. Particularly in U. S. A. and the Scandinavian countries it has been used in the clinic as a valuable instrument for a variety of respiratory function tests. In the instrument, which measures the nitrogen undisturbed by common respiratory gases, that is: oxygen, carbon dioxide and water vapor, the gas to be analysed is exposed to an electrical excitation which causes the gas to glow. By using the proper filter the spectroscopic bands in this glow belonging to the nitrogen can be separated and the intensity of these nitrogen bands can be measured with a photocell amplifier.

In our laboratory we have been working with nitrogen elimination from body tissues (Lundin 1953). For that reason we have made quite a few modification on the original Lilly's Nitrogen meter to obtain greater sensitivity and better stability. When using a DC amplifier as in the original Lilly Nitrogen meter there will always be a little zero displacement, which makes a repeated control of the zero point necessary. We therefore decided to make a Nitrogen meter with an AC amplifier. To make this possible we tried to shop the output from the photocell, but had some difficulties. In our latest model we shop the light beam from the ionization tube as will be described later. We now have an instrument with all the properties of the original model, but which can be directly connected either to a galvanometer or to an inkwriter. This makes the instrument very valuable for clinical use in pulmonary function tests which include analysis of nitrogen. Lung volume determinations, measuring of nitrogen washing out time during oxygen breathing and form of the nitrogen elimination curve can be easily obtained with this new instrument. Particularly valuable is the possibility to use it with an inkwriter. This makes it possible to follow the course of an experiment continuously and in case of air leakage during oxygen breathing to stop the experiment and repeat it with a minimum of time lost.

Description of the instrument.

Fig. 1 shows a schematic picture of the instrument, Fig. 2 electric circuit of the analyser.

The gas mixture to be analysed is continuously drawn through a needle valve (Fig. 3) at a speed dependent on the pressure in the Nitrogen meter system. With the pump used (Cenco Hyvac) and the pressure 2 mm Hg

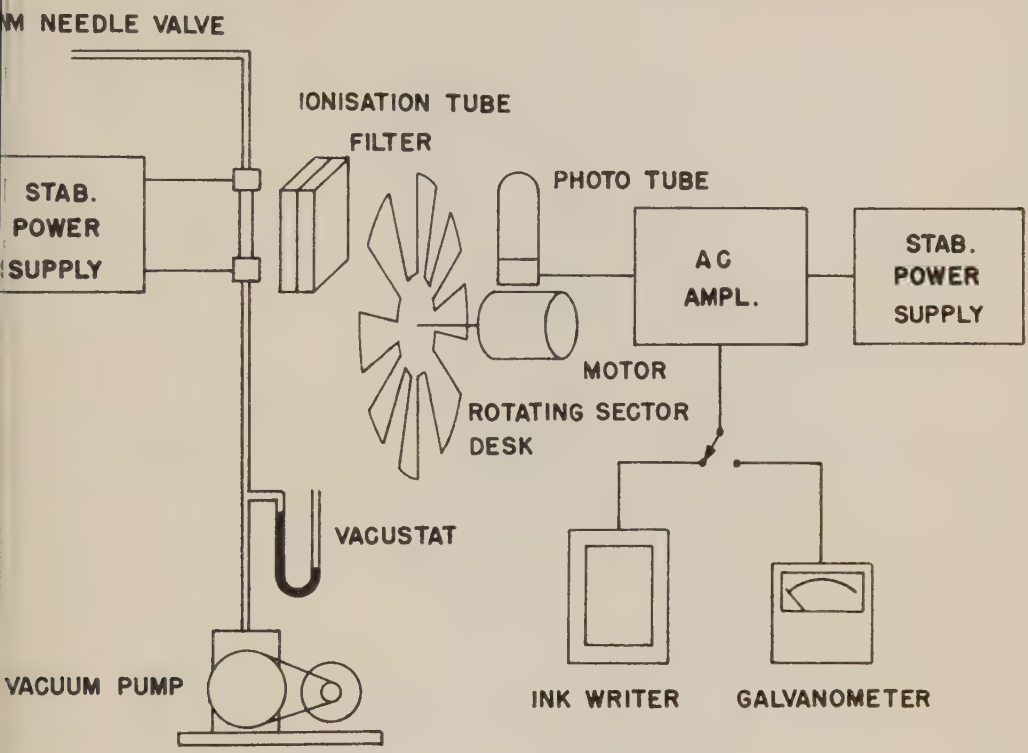

Fig. 1.

ut 20 ml gas per minute is drawn into the trument. In the tubing system the gas npled is transported through the ioniza- n chamber, a pyrex tube 15 mm in length l with a bore of about 1 mm. This tube is ed at both ends into cylindrical electrodes, which a high voltage, 900 V, stabilized ect current is applied from a power ply. This ionization current can be chosen ween 3—5 MA. The light from the ioniza- n tube is modulated at a frequency of 800 by a rotating sector desk, and after pas- g through a pair of filters, Corning 5850 Corning 428, it activates a photocell. e sector desk is shaped to give the light falling upon the photocell a shape near sinus wave. Consequently the voltage output from the photocell is easily amplified by an AC amplifier, with a large amount of negative fed-back. It consists of two pentodes and a cathode follower with negative feed-back between the cathode follower and the cathode of the first penthode. It is followed by a buffer tube, another cathode follower, and a power penthode without cathode condenser giving negative current feed-back. The output from the power tube is taken through an output transformer and is rectified by a pair of germanium diodes to give the driving voltage for an Esterline Angus inkwriter

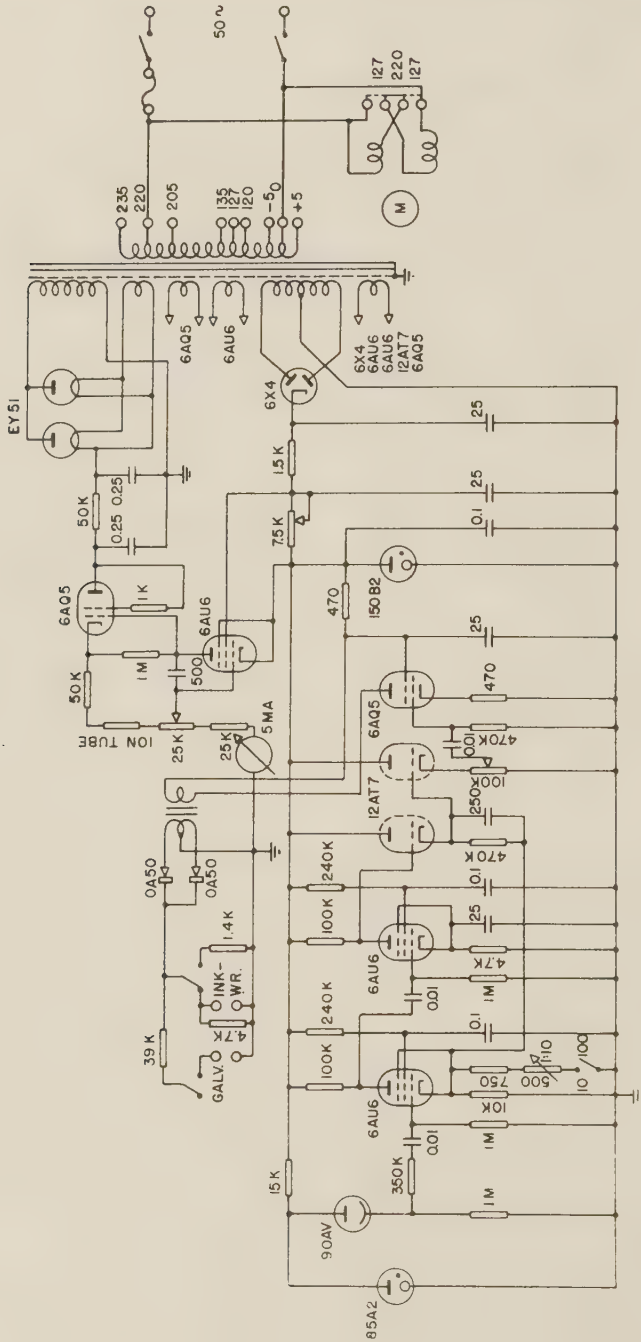
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Fig. 2.

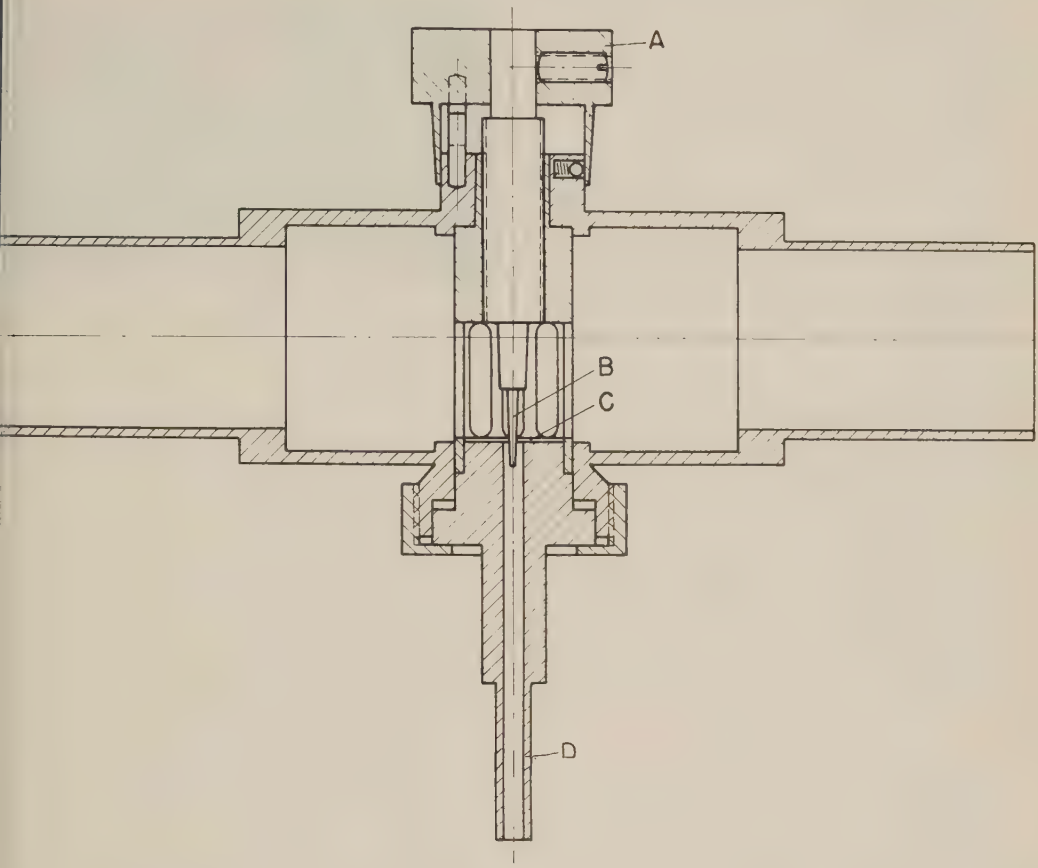

Fig. 3. A, knob with scale for adjustment of needle B in orifice C. D, outflow to Nitrogen meter.

mA, or a Cambridge galvanometer 240 uA. ne Esterline Angus is used to give a direct k record with an accuracy of 1 per cent and e Cambridge galvanometer for direct read- with an accuracy of 0.2 per cent. A switch nnects the desired instrument to the circuit. oth amplifier and photocell receive stabi- ed anode voltage from the power supply. compensate for the temperature drift of e photocell, an NTC-resistor is used in its cuit.

The Nitrogen meter has the following controls. On the front panel (Fig. 4), from the right, an on-off switch, a screw-driver control for the ionization current and a control instrument for this current. There is a knob control for normalization against air (air is used to give a calibration point). A switch allows shifting between two sensitivity ranges, one being 10 times as sensitive as the other. The range marked 0—100 is used

for higher nitrogen percentage measurements, that is around air, and the range marked 0—10 to measure nitrogen percentages between 0—10 per cent. For the adjustment of the 1 to 10 ratio between the two sensitivity ranges of the instrument, there is another screwdriver control.

On the back of the Nitrogen meter there are outlets to the recording instruments, that is a galvanometer and an inkwriter with a switch for the connection of the instrument to be used.

The instrument is calibrated against Haldane analyses. The shape of the calibration curve is very stable provided the same ionization pressure and the same vacuum pump and indicating instrument are used. The only calibration needed before an experiment is control of the air point and the 10 : 1 ratio between scale 0—10 and 0—100. Warming up time of the instrument is less than one hour.

Some ways to use the Nitrogen meter.

We think it worth while to mention a few ways in which the instrument can be used. Besides the lung function tests mentioned, where the alveolar air nitrogen concentration can be followed breath by breath, the instrument can be used in respiratory and metabolic experiments combined with a fast CO_2 analyzer (Fowler 1949) to follow all the respiratory gases. For instance the change in alveolar gas composition when shifting from rest to work, and from work to rest. We have also used the instrument in combination with a low pressure chamber to measure the change in alveolar air nitrogen during oxygen breathing, which should give

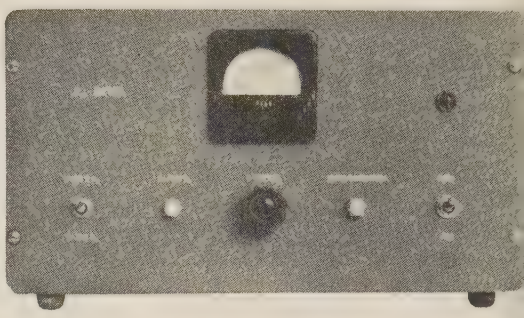

Fig. 4.

a picture of the tissue nitrogen elimination By working at lower atmospheric pressure the output of the Nitrogen meter can be raised several times for the same amount of nitrogen eliminated at ground pressure and with the same ionization pressure. This means that with an elimination of 1 ml nitrogen per minute, which at ground level corresponds to nitrogen percentage differences between inhaled oxygen and alveolar gas of about 0.02 per cent, at ¼ atm and with the CO_2 and H_2O absorbed before the alveolar gas reaches the ionization tube, the nitrogen percentage will rise to 0.14 per cent. The difference can be measured with an accuracy of about $\pm$ 7 per cent or $\pm$ 0.01 percentage point which is equivalent to $\pm$ 0.0015 per centage point at ground level. Thus we have a sensitivity far beyond that of any other instrument of this kind, which makes it, for the first time, possible to follow tissue nitrogen elimination for several hours with a very high accuracy.

SUMMARY

A development of the Lilly Nitrogen meter is described where the amplifier is converted to an AC system and the stability

improved. The instrument is particularly uable as a clinical instrument when com- ed with an inkwriter. An example is also en how to increase the output of the instrument 7-fold without raising the amplification, and thus make it possible to measure the elimination of very minute amounts of tissue nitrogen during oxygen breathing.

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ELECTROPHORETIC TECHNIQUE IN BACTERIOLOGY

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According to the current interpretation, the cytoplasm of the bacterial cell is surrounded by a limiting organ built up of several concentrically arranged layers. It is usual to make a rough distinction between the so-called cytoplasmic membrane which is the innermost layer, and the cell wall which is the outermost. In many cases, however, the cell is surrounded by a capsule which cannot be considered as part of the cell in the strictest sense, but rather as an accumulation of excreted products of high viscocity about to leave the cell. The capsule can be removed by washing without affecting the viability of the cell.

It has long been known that the surface layers serve not only as a simple covering for the liquid cytoplasm, but also carry out an extremely complicated, selective membrane function for the preservation of an optimal internal environment and for protection against noxious external influence. It is further known that a considerable part of the antigen apparatus of the cell is associated with the surface layers — such as the gram property of gram-positive bacteria. Important enzymes and activators are located on the surface, and a lively synthetic activity takes place here. Several of the most effective bactericidal substances known are effective by injuring the limiting organ of the cell and thus interfering with one or more of its vital functions.

It is natural that the interest of microbiologists in recent years has been directed increasingly toward the limiting organ of the cell since it is characterized by such prominent properties and its functional condition has proved to be of fundamental significance for the biologically normal cell. Special interest is associated with the processes localized to the actual surface of the bacterial cell, *i. e.* the border between the cell and the surrounding medium.

By various means some ideas have gradually been obtained as to the construction, composition and properties of that part of the cell which is exposed to its surroundings. One of the methods which has been employed is microscopic electrophoresis — often called microelectrophoresis — and which is known especially from Abramson, Moyer & Gorin's (1942) extensive monograph. This method has indirectly afforded certain information as to the nature of the bacterial surface and the changes which it may undergo as a result of physiological variations of the environment or injuries of various kinds.

A brief account of the application of electrophoretic technique in bacteriology for investigating the bacterial surface will be given, but first the electrophoresis phenomenon itself will be described.

Electrophoresis is an electrokinetic phenomenon which has been recognized for almost a century, and in connection with microbes for about 50 years. The phenomenon occurs when suspended particles with an electric surface charge are brought under the influence of an electric field. It can be observed when an electric current is passed through a colloidal system or a suspension of particles

n as bacteria or other cells in an aqueous lium. The particles will then migrate in direction of the cathode if they are posi- ly charged, or toward the anode if nega- ly charged. There are several factors ch contribute to the surface charge, some which predominate. If there are surface ponents with polar groups to any great nt, *e. g.* proteins, a dissociation of the r groups will be the main cause of the rge. In other cases, such as particles hydrophobic surfaces, the charge will lue primarily to a selective adsorption ns (usually anions) from the suspension lium to the particle surface. In all cases adjacent fluid will be oppositely charged, ng rise to an electric double layer around particle. This is the origin of the so- d zeta-potential.

he migration speed of the particles in an ric field is of primary interest because an expression of the strength of the elec- horesis phenomenon. The migration , however, is determined by a number ctors. In addition to the strength of the ric field, the chemical constitution of the icle surface and the nature of the suspen- electrolyte, its ionic strength and pH also be significant. Furthermore, the osity of the suspension medium and, sequently, also temperature will be im- ant. On the other hand, the shape of particles and their density in the suspen- are insignificant within wide limits. relation between the migration speed the above-mentioned factors is in some s very complicated and cannot be taken here.

or microscopically visible particles, the ration speed can be measured directly with relatively high precision with the of a so-called microelectrophoresis cell. principle the apparatus employed is rely different from that used for in- igating colloids. In the latter case papir trophoresis or Tiselius' apparatus — ch has also been constructed on a micro- e — is employed.

As already mentioned, the migration speed is related, among other factors, to the chemical constitution of the particle surface. It is possible to work out a standard technique so arranged that alterations in the chemical constitution of the microbial surface will be reflected in alterations of the migration speed. This can be achieved by keeping all of the other significant factors constant and under full control. It is primarily this procedure which has afforded possibilities for the application of electrophoretic technique in bacteriology, and which has formed the basis of a long series of investigations during the last 30 years. The earlier investigations, however, must be judged with some reservations as the microelectrophoretic technique was not adequately developed until recent years (cf. Moyer, 1936).

When microbes are the object of investigation, it must be remembered that their surface is not homogenous, but comprises a mosaic of different types of substances, each of which contributes in varying degrees to the surface charge . However, for practical reasons the charge is considered as evenly distributed. Care must also be taken that the media in which the bacteria are cultivated before they are examined electrophoretically do not contain components which can be adsorbed to the bacterial surface and are not removable by washing.

Modern microelectrophoresis cells are made entirely of glass and can be placed directly on the stage of the microscope (cf. Abramson's cell described by Moyer, 1936). The microbes to be examined are placed in the microelectrophoresis cell after having been carefully washed and then suspended in a suitable buffer solution with the proper ionic strength and pH. An electric current is passed through the cell *via* non-polarizing electrodes. The current can be accurately measured and kept constant during the registration. A sketch of the apparatus is shown in Fig. 1. Microbes are usually more or less negatively charged when suspended in a buffer with a pH around the neutral point.

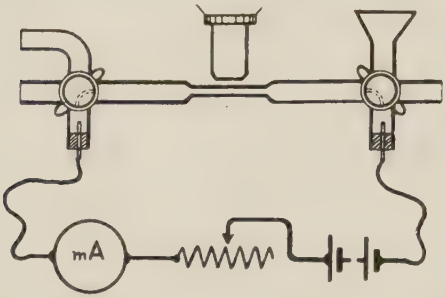

Fig. 1. Sketch of the microelectrophoretic apparatus. (Abrahamson's cell.)

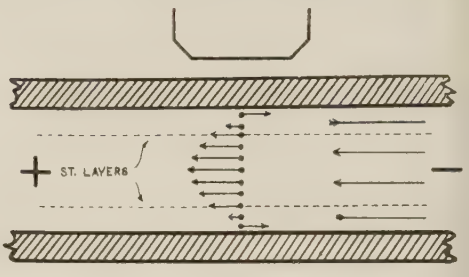

Fig. 2. Particle speed in different levels of t electrophoresis chamber.

They will therefore migrate toward the anode when the current is turned on.

The microbes are observed in the center of the microelectrophoresis cell — in the so-called central chamber — which is a thin, flat rectangular chamber with a depth of about 0.6 mm. When the focus of the microscope is shifted down through the central chamber, it will be seen that the speed of the particles is not the same at all levels, as illustrated in Fig. 2. This is due to the simultaneous occurrence of the electro-osmosis phenomenon. The internal walls of the chamber, because they are glass, will be negatively charged, while the adjacent fluid layer is positively charged. Consequently this fluid layer will migrate toward the cathode when the current is on. In the central part of the chamber there will be a compensatory reflux. The electro-osmosis phenomenon causes the observed particle speed to be a parabolic function of the depth of the chamber. There are, however, two levels where the observed migration speed is equal to that of the microbes in relation to the fluid. It is here that the focus must be adjusted during registration. The location of these stationary levels can be calculated mathematically and are close to 0.2 and 0.8 of the total depth of the chamber when this equals 1.

The speed measurements are made by recording with a stopwatch the time which a larger number of microbes (*e.g.* 50) require to cover a certain distance l (measured in μ marked on a micrometer scale in the ey piece. The average time t (in seconds) calculated. If the field strength during th experiment is designated X (given in vol cm), the results will be obtained in the for of the so-called electrophoretic mobility, according to the formula:

$$v = \frac{l}{t\ X}$$

in μ/sec. volt/cm. The field strength ma be calculated according to Ohm's law, tha

$X = \frac{I\ R}{q}$ where I is the strength of th current through the cell during the registra tion (measured in amp.), R the specif resistance in the suspension buffer (in ohr cm) and q the cross section of the centra chamber (in cm²). The results are alway given in relation to the buffer solution use as a suspension medium and are usually co rected to 25° C according to the formula:

$$v'_{25} = \frac{\eta}{\eta_{25}}\, v'$$

where v_{25} and v designate mobility at 25° and at the experimental temperature respe tively, and η_{25} and η correspondingly for th viscosity of the buffer solution.

On the basis of the mobility, the zeta-ential (ζ) on the surface of the particle be calculated with Helmholtz' equation:

$$\zeta = \frac{4\pi\eta}{D} v \qquad \text{(ESU — cgs)}$$

ere η is the viscosity of the suspension lium and D its dielectric constant. How-r, this has no practical advantages and eldom done.

The microelectrophoretic technique can applied according to various principles:

) As mentioned above, a standard regi-tion technique can be devised whereby factors are kept constant during all of registrations. A statistically significant iation between two mobility determina-s will then indicate that the average icle surface is not the same in the two ensions compared. With this technique possible to observe how different kinds experimental treatment of the microbes reflected in their electrophoretic behavior, rding a basis for certain conclusions. It ossible to register the effects of chemical nts which are known to react with certain ups or which injure the cell in a specific ner. It is also possible to register the ct of treatment with solvents or enzymes ch remove certain components from the face of the microbe. By comparison with effect on known model particles with ogenous surfaces, it has also proved pos-e to obtain valuable information on the ure of the microbial surface. With this nique it is furthermore possible to in-tigate the significance of all of the factors ch, in one way or another, are involved he growth requirements of the microbes rche, 1953).

new modification of this principle of stigation is given by Dyar & Ordal 46). They found that lipids can be de-ed and that their proportion of the robial surface can be calculated after a able detergent has been added to the suspension buffer in varying concentrations. They found that certain anion detergents were especially suitable, *e. g.* the sodium salts of duo-, tetra- and hexa decylsulfonic acids which have a sufficiently long hydrocarbon chain and a negatively charged group in one end in a state of ionization. The lipophile hydrocarbon chains will be attracted by any lipids present on the microbial surface, while the hydrophile polar groups will be oriented outward in the fluid and will increase the negative charge of the microbes. This situation will result in a measurable increase of the electrophoretic mobility of the microbes, indicating the presence of lipids on the microbial surface.

2) Electrophoretic technique may be employed to obtain information on the presence of dissociated, eventually amphoteric components on the microbial surface as these will cause the mobility to vary with the pH of the suspension buffer. At sufficiently low pH values, mobility should be reduced to 0 or reversed. The experiments are planned so that the mobility is registered in a series of buffers with the same ionic strength but with varying pH. All other experimental conditions are kept constant. It should be pointed out, however, that at pH values under 4 the microbes may be injured and undergo irreversible changes which detract from the value of the results.

3) Furthermore, mobility can be registered correpsondingly in a series of buffers with constant pH but with varying ionic strength, and otherwise under uniform experimental conditions. Inert particles with hydrophobic surfaces, whose charge is due mainly to a selective adsorption of anions from the suspension medium, behave in a particular manner under such conditions. If microbes are examined according to this principle, it will be possible to draw important conclusions regarding the nature of the microbial surface from their electrophoretic mobility.

SUMMARY

The electrophoresis phenomenon and the microscopic method of electrophoresis which can be employed in investigating microbes are briefly described. The basic principles for microelectrophoretic investigations c microbes are discussed, and the possibilitie which they afford for examining the natur of the microbial surface are indicated.

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NOTE ON THE DETERMINATION OF RADIOIRON*

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(Received for publication April 12, 1954)

hortly after radioiron became available, n, Bale, Lawrence & Whipple (1939) ied this radioisotope with much success ie study of the absorption and distribu- of iron in the animal body. An analytical od was devised by Hahn, Bale & Bal- (1942) which permits the determination ie iron content of a tissue fraction and radioactivity. Following wet ashing of tissue and after addition of a known nt of carrier the solution is electrolyzed the iron deposited on a copper planchet h is then placed under the Geiger ter. The yield of electroplating is tested issolving the electroplated iron in acid carrying out a colorimetric determina- of the iron content of the solution.

ough this analytical method proved to f great importance in radioiron studies, were induced to replace it by a much e rapid, simple, and certainly not less t one since a long series of radioiron rminations in numerous tissue fractions to be carried out.

he new procedure is based on a) colori- ic determination of the iron content of lution of a tissue fraction after wet g, b) addition of carrier iron as $FeCl_3$ ring up the iron content of the sample total amount of 500 micrograms, c) pre- ation of the solution with H_2S after ralizing it with ammonia, d) collection e FeS obtained on a filter paper placed he bottom of a perforated aluminum of 1.2 cm diameter and 2 mm depth

This work was supported by a grant from hs Medicinska Forskningsråd.

Table I. *Added and recovered radioiron Fe^{59} as ferrichloride was pipetted into test tubes, 500 micrograms of carrier iron added, and the procedure described in the text carried out.*

Fe^{59} added (counts per min.)	Fe^{59} recoveted (counts per min.)
10	10.2
20	19.7
30	29.2
100	98.0
200	200.0
300	294.0

Table II. *Serum from human subjects previously injected with serum containing Fe^{59}. 1 ml serum was dried at 100° C, wet ashed with 1 ml sulfuric acid and some perhydrol and its activity determined as described.*

Sample	Relative active units per ml of serum		
	Combustion 1	Combustion 2	Combustion 2
1	269	230	234
2	194	171	-
3	137	133	141
4	108	98	100
5	79	77	79
6	62	62	63
7	1.9	1.9	3.3
8	2.8	208	2.5
9	263	267	275
10	35	35	37
11	19	20	23
12	6.3	5 5	6.8
13	10	10	10

(as used in the determination of the radioactivity of ammonium magnesium phosphate precipitates), e) placing the aluminum dish under the Geiger counter. (Since the 500 micrograms of iron are about equally distributed over a surface of 1.2 cm^2, we are measuring the radioactivity of an almost infinitely thin iron layer. The intensity of the β-radiation emitted by Fe^{59} is reduced to half its value when passing a layer of 10.0 mg of aluminum per cm^2.

A few examples of the method described are given in Table I which contains data on added and recovered amounts of Fe^{59} obtained when preparing "standard" samples.

Since the procedure is rapid and easy to carry out and requires no special equipment but a Geiger counter, it can be used in most clinical laboratories. The accuracy obtained in clinical application of the method is seen from Tables II and III. Duplicate determinations involving both combustion and counting technique are stated.

SUMMARY

A simple and rapid method for the determination of Fe^{59} is described. The radioiron is precipitated as FeS and after filtration of the solution counted on the filter papers.

Table III. *Determination of Fe^{59} conter of hemin isolated from human erythrocyte Combustion as stated in Table II, iron deter mined colorine trically with sulfo- salicylic acid.*

Sample	Activity units per microgram iron	
	Combustion 1	Combustion 2
1	0.37	0.35
2	0.24	0.24
3	0.16	0.16
4	0.031	0.029
5	0.049	0.051
6	0.27	0.23
7	0.24	0.23
8	0.27	0.29

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Letters to the Editors

BACTERIAL PHAGOCYTOSIS OF LEUKOCYTES IN DISSEMINATED LUPUS ERYTHEMATOSUS

In the plasma of patients suffering from sseminated lupus erythematosus there is esent a factor that causes a l. e. cell phenoenon. It has also been found to reduce the idation-reduction activity of both homogous and foreign leukocytes (Shaudhuri Martin 1953). These investigators also und that the leukocyte migration of l. e. d. tients was smaller than for healthy indiduals — a difference that disappeared after o weeks of adrenal hormone therapy.

Because bacterial phagocytosis is one of e most important functions of the leukotes, we have made some phagocytosis tests a typical l. e. d patient, a patient upon hom the l. e. cell test has also been positive peripheral blood.

METHODS

Venous blood was defibrinated immediately after ing taken. Anti-coagulants could not be used cause they might have had some effect on the agocytosis. A common salt suspension of Staylococcus aureus was used as bacteria. This was ashed three times and prepared immediately bere use. Equal amounts of defibrinated blood and cteria suspension were taken in a Pasteur pipette, ter which the mixture was incubated for 15 mines at a temperature of 37° C. After incubation a ear preparation was made from the mixture and ained in the usual way according to May-Grünald-Giemsa.

RESULTS AND DISCUSSION

If the lymphocytes are disregarded, 90 per nt of the leukocytes of l. e. d. patients had early "phagocyted" the Staphylococcus reus strain used in the tests, in one cell

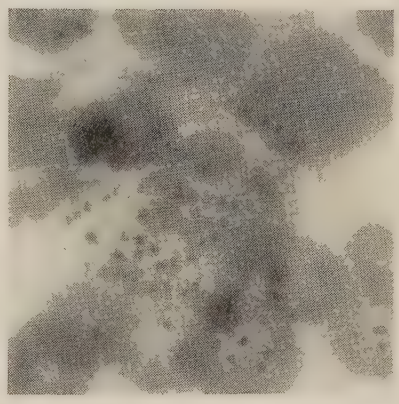

Fig. 1. L. e. cell with "phagocyted" bacteria in inclusion body.

there could well be some twenty bacteria. The percentage figure of the phagocyted leukocytes mentioned is normal. An interesting subsidiary phenomenon was that two l. e. cell-like formations were found in the preparations. In both the inclusion bodies there were abundantly phagocyted bacteria. One of them can be seen in Fig. 1.

Although, as mentioned earlier, the plasma of the l. e. d. patient has a decreasing effect on such an important biological occurrence as the oxidation-reduction activity of the leukocytes, this phenomenon, however, does not seem to have effect on the bacterial phagosytosis of the leukocytes in the l. e. d. patient.

It is a little difficult to explain the bacteria that we found contained in the inclusion

bodies of the l. e. cells. One possibility might be that they come from the bacteria which the leukocytes which took part in the formation of the l. e. cell phagocyted a little earlier. Against this, however, there is the fact that the inclusion body of the l. e. cell is considered to be of a nuclear origin (Lee, Michael & Vural 1951). Another possibility is that the phagocytosis might have happened first after the formation of the l. e. cell. When considering only this possibility it must be noted, however, that the l. e. cell is not regarded in a biological sense as living, but rather as a degenerate product, which because of the effect of the factor contained in the plasma, appears in stationary blood in vitro.

SUMMARY

The investigators have carried out tests in regard to the bacteria phagocytosis in the blood of a patient suffering from disseminated lupus erythematosus. 90 per cent of the leukocytes (excluding the lymphocytes) of the l. e. d patient had phagocyted the Staphylycoccus aureus strain used in the tests and this must be regarded as normal. The investigators also found 2 l. e. cell-like formations and here both their inclusion bodies contained bacteria in abundance (Fig. 1).

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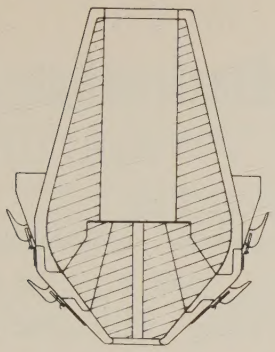

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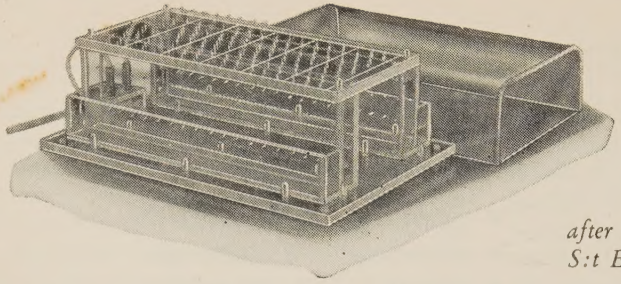

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